British Journal of Medicine & Medical Research
14(9): 1-32, 2016, Article no.BJMMR.24631
ISSN: 2231-0614, NLM ID: 101570965

SCIENCEDOMAIN *international*
www.sciencedomain.org

The Hsp90 Chaperone Machinery: An Important Hub in Protein Interaction Networks

Jürgen Radons[1*]

[1]*Scientific Consulting International, Altötting, Germany.*

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

Article Information

DOI: 10.9734/BJMMR/2016/24631
Editor(s):
(1) Crispim Cerutti Junior, Department of Social Medicine, Federal University of Espirito Santo, Brazil.
Reviewers:
(1) Jose Gutierrez-Salinas, Institute for Social Security and Services for State Workers, Mexico.
(2) C. K. Krishnan Nair, St. Gregorios Medica Research Centre, Kerala, India.
Complete Peer review History: http://sciencedomain.org/review-history/13840

Review Article

Received 27th January 2016
Accepted 2nd March 2016
Published 24th March 2016

ABSTRACT

Heat shock protein 90 (Hsp90) represents one of the most conserved proteins in living organisms and is present in all kingdoms of life except for Archaea. HSP90 proteins define a widespread family of molecular chaperones that play a fundamental role in protein homoeostasis and viability. HSP90s mediate folding and maturation of a broad spectrum of client proteins including steroid hormone receptors, transcription factors, and protein kinases. HSP90s primarily exist as homodimers whose activity is regulated by ATP. Hsp90 can adopt different ATP-triggered conformations, ranging from an open V-shaped unliganded to a closed ATP-bound state. HSP90 chaperones can be found not only in the cytosol, ER, chloroplasts, mitochondria, and the nucleus but also in the extracellular milieu where they act as potent stimulators of immune responses. The activity of Hsp90 is regulated by post-translational modifications and its association with numerous co-chaperones and client proteins involved in signal transduction and transcriptional regulation. Elevated levels of HSP90s can be found in a broad spectrum of cancers where they enhance cell growth, suppress senescence, and confer resistance to stress-induced apoptosis, including protection against cytostatic drugs and radiation therapy. Since numerous oncoproteins are clients of Hsp90, targeting Hsp90 represents a useful anti-cancer approach. In this review, the current knowledge on the Hsp90 chaperone machinery and its role in disease and therapy is compiled.

Keywords: Hsp90; regulation; function; therapeutic implications; disease relevance; steroid hormone receptor; inhibitors.

**Corresponding author: E-mail: j.radons@mail.de;*

ABBREVIATIONS

17-AAG: 17-allylamino-17-demethoxygeldanamycin; AD: Alzheimer's disease; ALS: amyotrophic lateral sclerosis; APC: antigen-presenting cell; CHIKV: Chikungunya virus; CHIP: C-terminal Hsp70-interacting protein; CRLM: colorectal liver metastases (CRLM); CTD: C-terminal domain; DC: dendritic cell; 17-DMAG: 17-dimethylaminoethylamino-17-demethoxygeldanamycin; EGCG: (-)-epigallo-catechin-3-gallate; EGFR: epidermal growth factor receptor; 5EPA: 5-episinuleptolide acetate; ER: endoplasmic reticulum; ERK: extracellular signal-regulated kinase; ESCC: esophageal squamous cell cancer; FGF: fibroblast growth factor; GA: gambogic acid; GDA: geldanamycin; Hip: Hsp70 interacting protein; Hop: Hsp70/Hsp90 organizing protein; HCV: hepatitis C virus; HSE: heat shock element; HSF: heat shock factor; HSP: heat shock protein; IGF-1R: insulin-like growth factor-1 receptor; Jak: Janus kinase; KSH: Kaposi sarcoma-associated herpes virus; MAPK: mitogen-activated protein kinase; MD: middle domain; MDS: myelodysplastic syndromes; MMP: matrix metalloproteinase; mTOR: mammalian target of rapamycin; NF-κB: nuclear factor kappa B; NK: natural killer; NSCLC: non-small cell lung cancer; NTD: N-terminal domain; PD: Parkinson's disease; PDGF: platelet-derived growth factor; PDGFR: PDGF receptor; PI3K: phosphatidylinositol-3 kinase; SHR: steroid hormone receptor; STAT: signal transducer and activator of transcription; TGF: transforming growth factor; TPR: tetratricopeptide repeat; TRAIL: TNF-related apoptosis-inducing ligand; VEGF: vascular endothelial growth factor.

1. INTRODUCTION

The 90 kDa heat shock proteins (HSP90s) are highly abundant and ubiquitous ATP-dependent molecular chaperones with diverse biological functions involved in maintaining normal tissue homeostasis. Similar to other HSPs, Hsp90 is capable of binding unfolded or non-native polypeptides and preventing their aggregation. Hsp90 was originally described amongst a defined set of heat shock proteins (HSPs) that are rapidly induced in fungal, plant and animal cells in response to acute thermal up-regulation [1]. It represents the major soluble protein of the cell and is most commonly located in the cytoplasm. Apart from their cytosolic localization, HSP90 paralogues can be found in the endoplasmic reticulum (ER), mitochondria, chloroplasts, and the nucleoplasma [2-4]. Moreover, HSP90 proteins can also be located on the cell surface and in the extracellular space, even though HSP90s do not bear a recognizable transmembrane domain for membrane anchoring. Although the first report of an ER/Golgi-independent release of Hsp90 from viable cells with intact cell membranes was made in the late 1980s by Hightower and Guidon [5], the molecular mechanisms underlying HSP release continue to be a matter of debate given that cytosolic HSPs lack a consensus signal for secretion. Different processes have been proposed, including non-classical exosomal release [6] and passive release after cell death by necrosis [7]. A wealth of evidence now demonstrates that membrane translocation and secretion of Hsp90 does not occur through the classical ER/Golgi protein secretory pathway [8,9].

1.1 The HSP90 Family of Chaperones

Eubacteria express a single Hsp90 homologue, referred to as HtpG (high-temperature protein G) that is absent in Archaebacteria with the exception of *Methanosarcina mazei* who possesses a gene that is well-aligned with the bacterial *htpG* [10,11]. HtpG is a dimeric phosphoprotein with functional and structural similarities to eukaryotic HSP90s. All eukaryotes have several HSP90-encoding genes leading to the expression of compartment-specific isoforms fulfilling organelle-specific functions. The human HSP90 family comprises five gene products differing from each other by expression level, subcellular location and amino acid constitution (Table 1). They are encoded by a multigene family encompassing six genes and 11 pseudogenes in humans [12]. Functional genes encoding HSP90 proteins map to several chromosomes as given in Table 1. The two major cytosolic HSP90s cover the inducible Hsp90α1 (Hsp90AA1, HspC1) and the constitutive Hsp90β (Hsp90AB1, HspC3), resulting from a gene duplication about half a billion years ago [13]. Hsp90α2 (HspAA2, HspC2) represents a putative shorter isoform of Hsp90α1 which was originally classified as a pseudogene. Nowadays, the existence of this protein is supported by unambiguous mass spectrometry evidence [14]. Hsp90α2 may be implicated in promoting the maturation, structural maintenance and proper regulation of specific target proteins.

Two additional homologues exist in mitochondria and the ER: Trap-1 and Grp94. Grp94 (HspC4, Hsp90B1) constitutes the ER paralogue of Hsp90 which arose by a gene duplication event very early in the evolution of eukaryotic cells [15,16]. Grp94 is present in all eukaryotes with the exception of fungi that have most probably lost it [15]. In contrast to heat-inducible Hsp90α, Grp94 is glucose-regulated and induced by glucose starvation [2]. It participates in protein folding and assembly, protein secretion, apoptosis protection, and mediating immunogenicity in tumour and virally infected cells [16,17]. Trap-1 (HspC5, Hsp90L) is located to mitochondria and contains a mitochondrial localization sequence at the N-terminus [3]. Trap-1 appears to represent a close relative of HtpG originating from a HtpG-like ancestor [3,11]. It is Involved in maintaining mitochondrial function and polarization and also acts as a negative regulator of mitochondrial respiration, able to modulate the balance between oxidative phosphorylation and aerobic glycolysis [18].

HSP90s function as part of multi-chaperone complexes by interaction with various co-chaperones that affect the binding specificity of Hsp90 for particular client proteins by promoting their conformational integrity. This multitude of regulatory interactors regulate the activity of the chaperone, making Hsp90 a central hub for several signalling pathways [19]. While Hsp90 ensures the stability of these client proteins, its inhibition leads to proteasomal degradation of the clients. Meanwhile, more than 400 clients have been identified up to date and many of them are implicated in mediating signal transduction pathways, apoptotic evasion, differentiation as well as metastasis [20-22]. The best characterized of the many client proteins (listed at http://www.picard.ch/) originate from two classes: steroid hormone receptors (SHRs) and protein kinases [23]. The range of Hsp90 co-chaperones also involves Hsp70 as well as Hsp40 (DnaJ), forming a major cytoplasmic chaperone network.

2. STRUCTURE AND FUNCTION

2.1 Structural Features

HSP90s are abundant and highly specialized molecular chaperones that primarily exist as ATP-regulated homodimers. Dimerization is essential for the vital functions of HSP90s [24]. Nevertheless, higher oligomeric states including hexamers have been reported [25]. It has been shown previously that oligomerization enhances substrate binding and prevents irreversible aggregation [26,27].

Almost all Hsp90 homologues display a common domain architecture with three well-defined domains: (i) a highly conserved N-terminal nucleotide binding domain (NTD) responsible for ATP binding and hydrolysis, (ii) a middle domain (MD) which completes the ATPase site necessary for ATP hydrolysis and binds client proteins, and (iii) a highly conserved C-terminal dimerization domain (CTD) with the pentapeptide M-E-E-V-D involved in the binding of several co-chaperones and other HSPs bearing a tetratricopeptide repeat (TPR) domain [28]. Members residing in certain subcellular compartments harbour an N-terminal localization signal, while the ER-specific Grp94 (HspC4) contains the C-terminal ER retention signal K-D-E-L [29]. The ATP-binding pocket within the NTD further functions as binding site of numerous natural substances such as the antibiotics radicicol and geldanamycin that promote degradation of protein kinases and interfere with Hsp90 functions [30,31]. In eukaryotes, a short charged region of about 50 amino acid residues links the NTD and the CTD [32]. This region varies in both, length and composition among species and isoforms with being entirely absent in mammalian Trap-1 and bacterial HtpG [32]. Although the charged linker is not essential for Hsp90 function, it is crucially involved in the coordination of the NTD and CTD to maintain the conformation of ATP binding to Hsp90. This inter-domain charged linker has been found to regulate Hsp90 allosteric signalling in conjunction with the MD [33].

Hsp90 dimers are extremely dynamic and plastic molecules whose chaperone activity has been linked structurally to a “molecular clamp” [34]. A considerable mobility of the molecular chaperone was seen in diverse structural arrangements of the crystallographic conformations, ranging from a structurally rigid and closed ATP-bound form of yeast Hsp90 (Fig. 1A, [28]) to a V-shaped apo-form (Fig. 1D+E) and a semi-closed ADP-bound form (Fig. 1C) of the bacterial homologue HtpG [35], and an intertwined conformation with close contacts between the two NTDs in the Grp94 homologue (Fig. 1B, [36]). In the nucleotide-free open state, the C-termini of two Hsp90 monomers interact thereby forming an anti-parallel V-shaped homodimer. Concurrently, the N-termini of the Hsp90 homodimer preserve an open-state facilitating the capture of client proteins [37]. ATP binding to the open structure

induces conformational rearrangements of the N-termini, resulting in closing of a “lid” over the bound nucleotide followed by the association of the NTDs. Continued rearrangements allow interactions of the NTDs and MDs, culminating in the closed conformation that is able to hydrolyze ATP [28]. ATP hydrolysis provokes opening of the lid followed by dissociation of the NTDs and subsequent ADP release, thereby returning Hsp90 to the open unliganded conformation [36]. It is still unclear to which extent the nucleotide state alone is able to define specific conformational states, since the Hsp90 homodimers have been found to exist in a dynamic equilibrium between open, closed and intermediate conformations [33]. In this regard, several client recruiter co-chaperones such as Rar-1 (required for Mla-12 resistance) and Sgt-1 (suppressor of G2 allele of SKP1) have been demonstrated to orchestrate global changes in the Hsp90 dynamics and stability, affecting ATPase activity and recruitment of client proteins [38].

2.2 Function of Intracellular HSP90 Chaperones

HSP90s define a widespread family of molecular chaperones that play a fundamental role in protein synthesis, folding and degradation. The functions of the different HSP90 family members depend on their cellular localization. Intracellular residing HSP90s launch a rapid response to environmental stressors such as heat, hypoxia, UV and gamma-irradiation, reactive oxygen intermediates (ROI), and injury-induced growth factors [40]. HSP90s are abundant and highly specialized ATP-dependent molecular chaperones essential for the integrity of multiple signalling pathways that are associated with cell proliferation and viability. There have been a number of particularly interesting and important findings relating to the role of Hsp90 in promoting and maintaining the proper assembly of multi-protein complexes including the kinetochore, PI3K-related protein kinase (PIKK), RNA polymerase II, snoRNA, RNA-induced silencing complex (RISC), telomere complex, and the 26S proteasome. To fulfill these certain duties, HSP90s mediate complex assembly and changes in the composition of the complex without being part of the final assembled complex [41]. Under stress, HSP90s prevent denaturation and aggregation of substrate proteins and promote refolding of denaturated proteins in a large cytosolic complex denoted as the foldosome [2,42] (Fig. 2). In eukaryotes, HSP90s mediate extensive stress signal transduction including substrate activation as well as folding of steroid hormone receptors (SHRs), transcription factors, and protein kinases [34,43,44]. Another important finding is that Hsp90 appears to be involved in maintaining the monomeric state of the transcription factor HSF-1 under non-stressful conditions [45]. Moreover, HSP90s function as an important hub in a variety of protein interaction networks promoting tumour cell development [34,46-48]. In malignantly transformed cells, HSPs enhance cell growth, suppress senescence, and confer resistance to stress-induced apoptosis including protection against cytostatic drugs and radiation therapy [49]. Several oncoproteins have been identified as being targets of HSP90s, rendering Hsp90 inhibition a promising approach in anti-tumour strategies [50,51]. In recent years a wealth of evidence has been collected to demonstrate that inhibition of HSP90s contributes to degradation of many oncoproteins, thus expanding anti-cancer approaches [28,34,44,52].

As outlined above, HSP90s are crucially involved in the functional activation of SHRs. SHRs play diverse roles in human physiology such as responses to stress, apoptosis induction, regulation of differentiation, sexual development, and, in general, maintaining homeostasis. SHRs are nuclear receptors that act as ligand-activated transcription factors whose activity require the presence of numerous co-chaperones [53]. SHRs depend on the binding of Hsp90 for efficient loading of their steroid ligands. However, the concept of SHR activation continues to be a matter of debate and different mechanisms have been suggested [53-55]. Based on current knowledge, the following reaction cycle of SHR activation is proposed (Fig. 2). In the absence of the steroid hormone, SHRs reside in the cytosol bound to a complex of HSPs, chaperones and co-chaperones including Hsc70, Hsp90, and p23 [56]. This multi-protein complex is referred to as the foldosome [53]. In the early stage of foldosome assembly, SHRs are recognized by the Hsc70/Hsp40 chaperone complex in an ATP-dependent manner [57]; see Fig. 2. This complex is modified by the docking of the adapter protein Hop (Hsp70/Hsp90 organizing protein) to the open-state Hsp90 via its TPR domains to form the intermediate foldosome complex [58]. Intermediate stages may also involve binding of Hop to the ADP-bound form of Hsp90 [53]. The intermediate complex has been postulated to further contain the co-chaperone Hsp70 interacting protein (Hip) which augments

recruitment of Hsp90 and Hop to the foldosome complex [59]. Maturation of the foldosome complex is achieved by the addition of the immunophilins FKBP51 (FK506-binding protein 51) and FKBP52 followed by dissociation of Hsc70/Hsp40, Hip and Hop as a result of conformational changes in Hsp90 induced by ATP binding [55]. Consequently, the NTDs are closed thereby attenuating Hop's affinity for the complex [60,61]. This N-terminally dimerized Hsp90 shows high affinity for p23 that stabilizes the Hsp90-complexed SHR [62]. Recruitment of p23 is facilitated by the Hsp90 ATPase activator Aha-1 which associates with the MD of Hsp90, thus contributing to SHR maturation [63]. The SHR now binds the steroid hormone [64], leading to conformational rearrangements that culminate in the nuclear translocation, activation and release of the SHR, followed by receptor dimerization and binding to response elements in regulatory regions of certain target genes [65, 66]. Studies probing SHR import revealed an active contribution of foldosome constituents to SHR nuclear translocation [67]. Further co-chaperones such as Bag-1, Hsp10, Hsp27, and Hsp60 have been reported to have nuclear effects on SHR actions. These molecules play a crucial role in SHR signalling as they associate with the foldosome complex without being directly involved in its assembly [53]. It is still enigmatic how the SHR nuclear translocation is regulated. It has been suggested that hormone-directed recruitment of dynein to FKBP52 might cause transport of the mature SHR complex to the nuclear compartment [68]. Notwithstanding this issue, further investigations are required in order to shed light on the role of single co-chaperones in Hsp90-mediated SHR regulation.

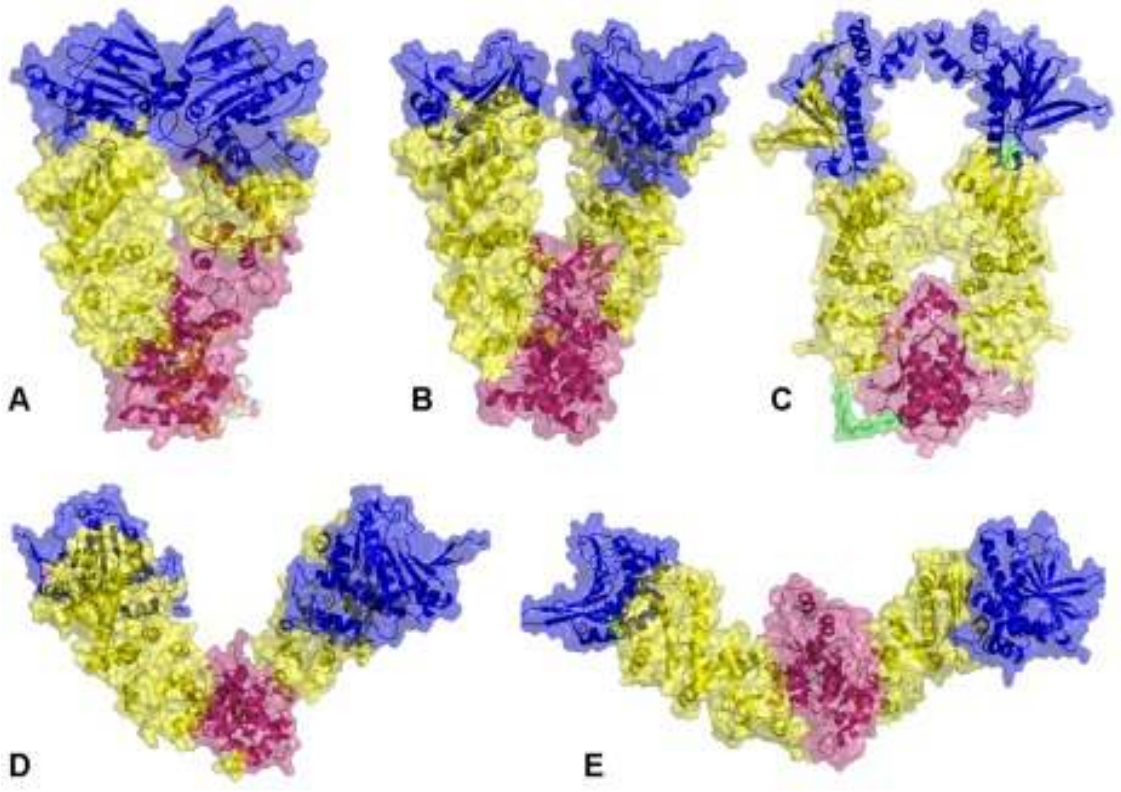

Fig. 1. Structures of the full-length Hsp90 dimer

Hsp90 can adopt different nucleotide-triggered conformations: (A) a closed ATP-bound conformation [28]; (B) a V-shaped conformation [36]; (C) a semi-closed, ADP-bound conformation [35]; (D) an open apo-form [35]; and (E) an extended apo-HtpG conformation [39].The NTD is given in blue, the MD is in yellow-green and the CTD in pink. The Hsp90 crystal structures are shown as ribbon representation overlayed with the surface representation at 50% transparency (reproduced from Dixit et al. 2012 [33])

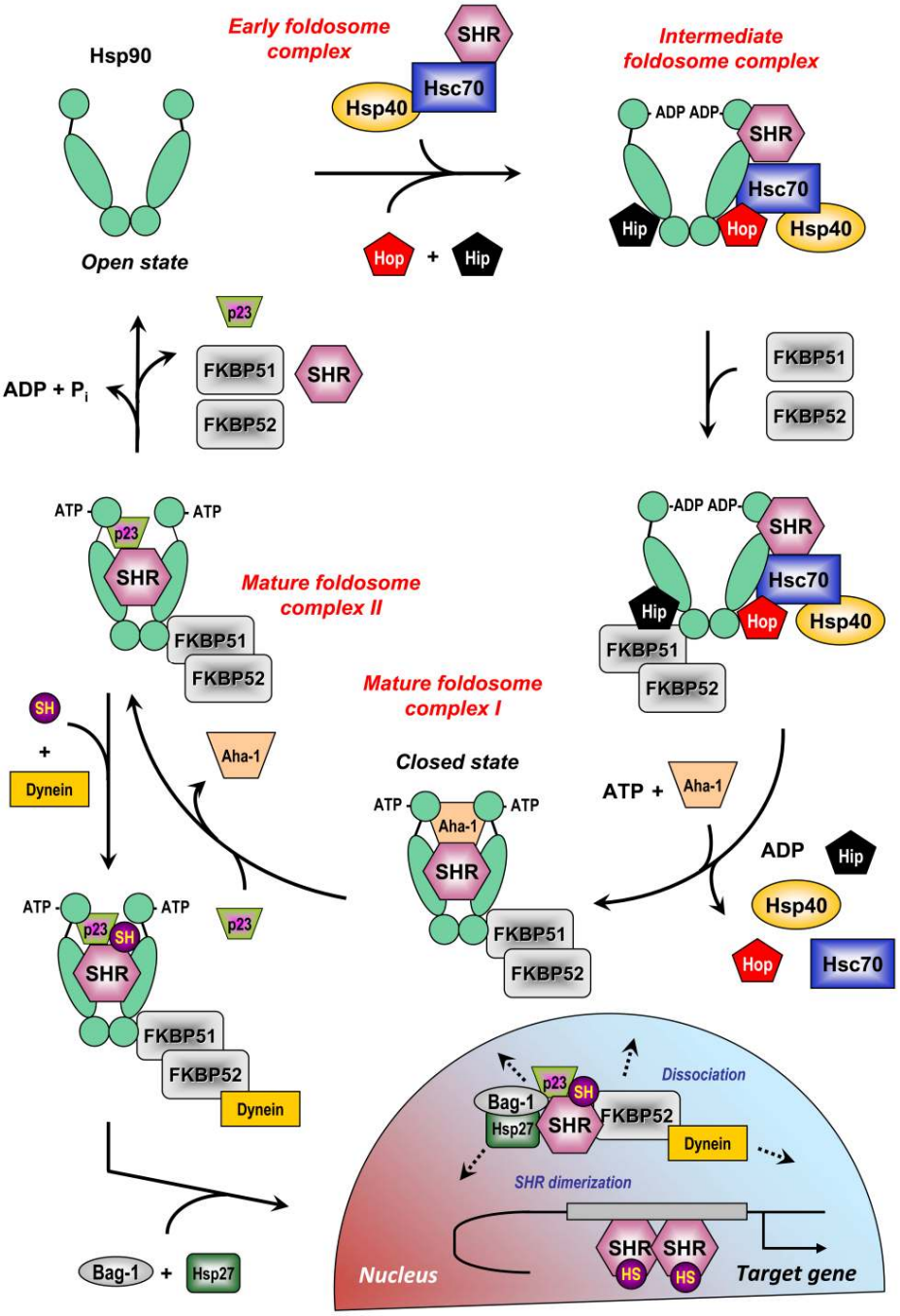

Fig. 2. The ATPase-driven steroid hormone receptor activation cycle

Schematic representation of previous work outlining the proteins involved in hormonal activation of the steroid hormone receptor (SHR). It is well accepted that three individual stages of foldosome assembly co-exist in the cell in a constant loop of association and dissociation: early, intermediate and mature. The model presented comprises recruitment of the Hsc70/Hsp40 chaperone complex to the SHR as a first step in foldosome assembly in the cytosol, followed by successive recruitment of numerous adapter molecules. This functional sequence is completed by concomitant recruitment of dynein and SHR translocation to the nucleus as a complex prior to the final dissociation of the complex within the nucleus to form the DNA binding-competent form of the receptor. The hormone-liganded dimerized receptor is primed for interaction with hormone response elements in regulatory regions of certain target genes; for details see text

2.3 Function of Extracellular HSP90 Chaperones

Evidence has emerged to demonstrate that normal cells secrete Hsp90 unless under environmental stress cues, whereas Hsp90 secretion occurs constitutively in certain tumour cells. Constitutive Hsp90 secretion has been reported as being linked to abnormalities in tumour suppressor genes and proto-oncogenes (summarized by Li et al. [69]). A few up-stream regulators of Hsp90 secretion in normal and tumour cells have been identified including p53 [70], the ubiquitine ligase Hectd-1 [71] and HIF-1α [72,73]. Amongst these, HIF-1α functions as a central regulator of the Hsp90 release. The studies by Li and colleagues convincingly reveal that HIF-1α mediates hypoxia-triggered Hsp90α secretion in primary human dermal fibroblasts and keratinocytes. A dominant negative mutant of HIF-1α has been found to inhibit Hsp90α secretion, whereas a constitutively active mutant of HIF-1α enables Hsp90α secretion even under normoxia; a mechanism that also may occur in tumour cells [72,73].

Extracellular HSP90s (eHSP90s) are considered as molecules with immunomodulatory functions, *inter alia*, through their ability to bind antigenic peptides during intracellular antigen processing. After their release from tumour cells into the extracellular compartment, HSP90/peptide complexes bind to surface receptors on antigen-presenting cells (APCs), followed by cross-presentation to $CD8^+$ cytotoxic T lymphocytes on MHC class I molecules culminating in specific tumour cell killing [74-76]. A wealth of evidence demonstrates that extracellular HSPs can stimulate cellular cytokine synthesis with the generation of pro- and/or anti-inflammatory cytokine networks regardless of chaperoned peptides. In the innate or peptide-non specific outcome, HSPs engage surface receptors that trigger the secretion of inflammatory cytokines from APCs via NF-κB activation and up-regulation of co-stimulatory molecules (*e.g.*, MHC II, CD86), followed by migration of dendritic cells (DCs) to draining lymph nodes [77]. The expression of a number of putative HSP90 receptors including scavenger receptors (*e.g.*, SR-A, CD36, SREC-1), the C-type lectin receptor CD91 (α_2-macroglobulin receptor, Lrp-1), and TLR-2/-4 have been identified on a range of cell types, some of which (*e.g.*, TLR-2/-4) have been suggested to facilitate the uptake of exogenous HSP90s and modulate the phenotype and function of APCs and T cell sub-populations, as well as the nature and potency of innate and adaptive immune responses. However, the function of these molecules as HSP90 receptors should be perceived with great care because of reports suggesting that the pro-inflammatory actions of HSP90s might rely on the binding of LPS which also interacts with these receptors, even though much of the evidence argues against this concept. Notably, cytokines themselves are able to modulate the synthesis of selected cell stress proteins and may also promote their release [78]. In a recent report, UV-radiation and cisplatin treatment rapidly induced the expression of membrane-bound Hsp90 (mHsp90) and promoted the release of IL-6 and IL-1β as well as DC maturation [79]. mHsp90 could facilitate the uptake of dying cells by bone marrow-derived DCs via the lectin-like oxidized LDL receptor-1 (LOX-1). In addition, mHsp90 was noted to promote the cross-presentation of ovalbumin antigen, and inhibition of the uptake of dying cells by LOX-1 decreased the cross-presentation of cellular antigen. From these findings it can be concluded that the rapid exposure of HSPs on dying cells at the early stage facilitates the recognition by and confers an activation signal to the immune system [79]. It is interesting to note that tumour-derived chaperone-rich cell lysates (CRCLs) containing Hsp90 and Grp94 have been identified to directly stimulate pro-inflammatory cytokine and chemokine production by NK cells, which may lead to activation and recruitment of macrophages at the tumour site, thus providing further insight into the function of CRCLs in anti-cancer immunity [80]. Experiments with pharmacological HSP90 inhibitors revealed a contribution of the transcription factor NF-κB, the oncogene AKT and the IκB kinase (IKK) complex in immune-stimulated production of inflammatory mediators such as IL-1, IL-6, TNF and NO [81,82]. Since the IKK complex plays a crucial role in carcinogenesis, the inhibition of its long-term activation by HSP90 and/or HSP70 modulators may prevent cancer development during chronic inflammation, one of the hallmarks of cancer.

A well-characterized of the many functions of eHsp90 is to promote cell motility, a central event in wound healing and cancer. eHSP90s play a central role in driving a non-motile tumour cell to become motile and invasive, as Hsp90 affects any step in tumour invasion including tumour cell migration [83]. The widely expressed cell surface receptor CD91 (Lrp-1) was identified by Cheng and co-workers as the receptor for eHsp90

involved in promoting cell migration [84]. eHsp90 may thus act as an autocrine and paracrine factor, promoting cell motility not only for tissue repair but also for tumour invasion and metastasis. In many tumour types, HIF-1α is constitutively activated and triggers Hsp90 release even in the absence of any environmental stress insult (see above). Tumour-derived eHsp90 then promotes tumour cell motility by interacting with either CD91 or other targets including MMP-2 [85], MMP-9 [86] and the Her-2 tyrosine kinase receptor [87].

3. REGULATION

3.1 Transcriptional Regulation

When cells are subjected to environmental stress, they respond by enhancing expression of HSPs. The rapid induction of HSPs in response to environmental stress is based on a variety of genetic and biochemical processes referred to as the heat shock response (HSR) [88]. The HSR is an ancient and sophisticated cytoprotective mechanism to augment organismal survival and longevity in the face of proteotoxic stress from without and within [89]. The HSR is highly significant in human pathology, as HSP levels increase in cancer and promote tumourigenesis and decline in protein aggregation disorders such as Alzheimer's disease [90,91]. The expression of at least two members of the HSP90 family is induced by stress (Table 1). HSR is regulated mainly at the transcription level by heat shock factors (HSF). Among them, HSF-1 is considered as being the key transcription factor of stress-inducible HSPs [92]. Under non-stress conditions, HSF-1 exists as an inactive monomer in the cytoplasm in association with Hsp70 and Hsp90 [93]. In response to stress, Hsp70 and Hsp90 proteins are released followed by the formation of phosphorylated and sumoylated HSF-1 homotrimers capable of binding to heat shock elements (HSEs) up-stream of *HSP* promoters, thereby triggering *HSPC* transcription [94, 95]. The mammalian target of rapamycin kinase (mTOR) plays a key role in response to proteotoxic stress due to its capacity to directly phosphorylate, and thus activate, HSF-1 [96]. Proteotoxic stress often results from the mTOR-mediated over-production of cellular components contributing to several processes that might become pathological such as cellular senescence, adipogenesis and glucose homeostasis.

The HSF-1 activity is negatively regulated at multiple levels. In this regard, activated HSF-1 trimers have been identified to interact with Hsp70 and the co-chaperone Hsp40 (DnaJ), leading to the blockage of its transactivation capacity [97]. HSF-1 transcriptional activity is also blocked by preventing DNA binding through acetylation of the DNA-binding domain of HSF-1. Moreover, the deacetylase and longevity factor SIRT-1 regulates the attenuation phase of the HSR by maintaining HSF-1 in a deacetylated, DNA binding-competent state [98]. As already mentioned, constitutive high levels of Hsp90 are frequently observed in cancer cells, in which the chaperone confers resistance to stress-induced apoptosis, serves in suppressing default senescence, and is associated with the development of metastasis and drug resistance. In tumours, Hsp90 may be also expressed irrespective of HSF-1 transcriptional activity. Possible candidates for Hsp90 synthesis in the absence of stress include the transcription factors NF-IL6, STAT-1, and STAT-3. As shown by different groups, IL-6 up-regulates Hsp90 and activates the Hsp90β (*HSP90AB1*) promoter via NF-IL6 (C/EBPβ) and STAT-3 [99,100]. Both factors bind to promoter sequences that are different to those that are used by HSEs and compete with HSF-1 for HSP90 expression on stress [99]. In contrast, STAT-1 recognizes IFN-γ activated sequences without competing with HSF-1 for transcription [101]. The effect of IFN-γ/STAT-1 is mediated via an Hsp90β (*HSP90AB1*) promoter region which also recognizes NF-IL6, STAT-3 and HSF-1; all of them acting together in up-regulating *HSPC* transcription regardless of stress [101]. In addition to SIRT-1, recent investigations identified mTORC-1 as a putative cancer-related HSF-1 regulator in tumours, contributing to Hsp90 over-expression by stimulating HSF-1 expression and/or activity [96]. In sum, these studies led to the identification of a composite response element within the *HSPC* proximal promoter region that connects the HSF-mediated HSR with IL-6 and IFN-γ signalling to regulate HSP expression. It has been shown previously that several inflammatory mediators and signalling molecules such as NF-κB and TNF are strictly linked to HSP gene expression and protein functions. In this context, the NF-κB subunit p65/RelA serves as a transcription factor for various HSPs including Hsp90 that in turn may have anti-apoptotic functions in cancer cells [102,103].

Table 1. The human HSP90 family of chaperones

Protein	UniProt ID	Aliases	Cellular localization	Length (aa)	Gene	Chromosome	Gene ID	Inducible
HspC1	P07900	Hsp90α1, Hsp90AA1, HspCA, Hsp86, Hsp89, Hsp90A, renal carcinoma antigen NY-REN-38	Cytosol, nucleus, cell membrane, extracellular exosomes	732	*HSP90AA1 HSPC1*	14q32.33	3320	Yes
HspC2	Q14568	Hsp90α2, Hsp90AA2, HspCA, Hsp90α-like 3	Cytosol, extracellular exosomes	343	*HSP90AA2 HSPC2*	11p14.1	3324	?
HspC3	P08238	Hsp90β, Hsp90AB1, Hsp84, HSP90B, HspCB	Cytosol, nucleus, cell membrane, extracellular exosomes, mitochondrion	724	*HSP90AB1 HSPC3*	6p12	3326	No
HspC4	P14625	Endoplasmin, Grp94, Gp96, Hsp90B1, Tra-1	ER, cell membrane, extracellular exosomes	803	*HSP90B1 HSPC4*	12q24.2-q24.3	7184	Yes
HspC5	Q12931	Trap-1, Hsp75, Hsp90L	Mitochondrion, nucleus, extracellular exosomes	704	*TRAP1 HSPC5*	16p13.3	10131	No

3.2 Post-transcriptional Regulation

Apart from its transcriptional regulation, HSP90 proteins have also been found as being regulated at the post-transcriptional level by micro-RNAs (miRNAs) that are critically involved in transformation, differentiation and proliferation. Global alterations in miRNAs can be observed in a number of disease states including cancer [104]. However, little information is available on the role of miRNAs in the regulation of the *HSPC* expression. The group of Biao Cheng identified Grp94 (Hsp90B1) as being a direct target of miR-223 in osteosarcoma, acting as a tumour suppressor via the PI3K/AKT/mTOR pathway [105]. A recent study convincingly demonstrated that hyperthermia-induced up-regulation of Hsp90 was suppressed by miR-27a in human oral squamous cell carcinoma cells [106]. In experimental cardiomyopathy, Hsp90β has been identified as an indirect target of miR-499 by modifying the phosphorylation state of Hsp90β [107]. More recently, miR-27b [108], miR-134 [109], and miR-222* [110] have been characterized as regulators of the HSP90 expression. However, future approaches analyzing the regulatory potential of miRNAs in the HSP90 expression will shed light on the post-transcriptional regulation of these chaperones.

3.3 Post-translational Regulation

Hsp90 is subjected to several post-translational modifications including phosphorylation (all isoforms), acetylation (Hsp90α1, Hsp90β, Trap-1), oxidation (Hsp90α), nitration (Hsp90β) and S-nitrosylation (Hsp90α1, Hsp90β) [111] as well as methylation (Hsp90α), N/O-glycosylation (Grp94, Hsp90β), ubiquitination (Hsp90α, (Hsp90β) and sumoylation (Hsp90α, (Hsp90β). Phosphorylation was the first identified Hsp90 post-translational modification in mammalian cells, affecting its function and interaction with client proteins including Aha-1, Cdc-37 [112], $pp60^{v\text{-}src}$ [113] and eNOS [114]. However, the influence of Hsp90 phosphorylation on its activity is far from being completely understood. Recent investigations by Wang *et al.* demonstrate that the phosphorylation status of Thr90 determines secretion of Hsp90α [115]. Evidence for an acetylation of Hsp90 has emerged after the discovery of the histone deacetylase 6 (HDAC6) as being an interaction partner of Hsp90 [116]. Hsp90 is known to be hyperacetylated at eleven lysyl residues culminating in blockage of ATP binding and affecting its interaction with several client proteins such as the glucocorticoid receptor [117,118]. The identification of HDAC1 and HDAC10 as further enzymes capable of deacetylating Hsp90 [119,120] reveals reversible acetylation as being a unique mechanism that regulates the Hsp90 chaperone complex activity.

S-Nitrosylation and oxidation are further post-translational modifications of Hsp90, affecting ATPase activity and affinity to client proteins [121,122]. Methylation represents a critical step in the post-translational modification of HSP90s. The only known Hsp90 methyltransferase identified so far is Smyd-2, and this enzyme is responsible for methylation of several lysyl residues in human HSP90 [123]. Smyd-2-dependent Hsp90 methylation has been shown to promote cancer cell proliferation by regulating the Hsp90 chaperone complex formation [124]. These data reveal a novel mechanism for human carcinogenesis via Hsp90 methylation which might allow the development of novel anti-cancer strategies. Selective and limited tyrosine nitration of Hsp90 plays a causal role in the induction of cell death [125], as nitrated Hsp90 binds and activates the ATP-gated ion channel P2X7, thereby eliciting apoptosis, *e.g.*, in motoneurons [125]. The toxic form of nitrated Hsp90 is detectable in pathological conditions such as amyotrophic lateral sclerosis (ALS) and spinal cord injury, rendering nitrated Hsp90 a potential diagnostic and therapeutic target.

Post-translational modifications of Hsp90 also include N/O-glycosylation as well as sumoylation. Asymmetric sumoylation of conserved lysyl residues in yeast Hsp82 and human Hsp90α promotes both, interaction with the co-chaperone Aha-1 and binding of HSP90 inhibitors [126]. Interestingly, cellular transformation is accompanied by elevated steady-state N-domain sumoylation, and increased Hsp90 sumoylation sensitizes eukaryotic cells to HSP90 inhibitors [126]. Human Hsp90α is also subjected to ubiquitination and acetylation. The ubiquitin ligase Hectd-1 has been found to poly-ubiquitinate Hsp90α, thereby affecting its intracellular location and blocking its secretion [71]. The ubiquitin ligase CHIP has been noted to ubiquitinate human Hsp90β, thus enabling the formation of poly-ubiquitin chains with Hsp70 [127]. These data highlight the mode of CHIP-mediated Hsp70/Hsp90 ubiquitination, a prerequisite for their proteasomal degradation.

4. CLINICAL SIGNIFICANCE

4.1 HSP90s and Cancer

There is a wealth of evidence indicating the significance of Hsp90 levels in tumourigenesis which may act as a potential tumour biomarker. Tumours often express high levels of catalytically active Hsp90 found in complexes with oncogenic client proteins, suggesting its role in survival and growth of malignant cells. Hsp90 is not generally up-regulated in cancer although its basal expression is already high in the majority of cells. Hsp90 is constitutively over-expressed in breast tumours, lung and gastric cancer, leukaemia (*i.e.,* myelodysplastic syndromes and acute myeloid leukaemia), renal cell carcinoma, melanoma, endometrial cancer, and Hodgkin lymphoma (reviewed by Ciocca and Calderwood, 2005 [128]), and thus it contributes to tumourigenicity and cancer cell resistance. HSP90s act through both its anti-apoptotic role and its chaperone function of stabilizing many kinases involved in cancer cell signalling, including tyrosine kinases (*i.e*., FLT3, Jak-2, v-Src) and serine/threonine kinases (AKT, Raf-1); for a review see Sevin et al. [129]. With respect to melanoma, HSP90 expression was significantly higher in tumours than nevi and correlated with disease progression, rendering Hsp90 a valuable drug target and useful diagnostic marker in this cancer entity [130]. A positive correlation between the Hsp90 expression and higher tumour grade has been reported in hepatocellular carcinoma (HCC) [131], bladder cancer [132], and epithelial ovarian carcinomas indicating that it might be a reliable indicator of aggressiveness [133]. In head and neck carcinoma, high levels of Grp94 in tumour tissues significantly correlated with advanced cancer stages and poor survival [134]. Hsp90 is also over-expressed in prostate cancer cells compared with normal prostate tissue and thus provides a potential selective target [135]. However, its role as a predictive marker in prostate cancer remains unclear since several contradictory results exist in this regard [136,137]. Meanwhile, Hsp90 plasma levels have been positively correlated with tumour malignancy in clinical cancer patients. In this respect, plasma Hsp90α was significantly enhanced in patients with malignant tumours of breast, lung, pancreas, and liver in comparison with normal people and patients with benign tumours [115]. Moreover, the levels of plasma Hsp90α in liver or breast tumour patients with metastasis were dramatically up-regulated compared to those without metastasis, providing further evidence for the association of eHsp90α with tumour malignancy and metastasis.

It has been reported that Hsp90 inhibition reduced cell motility and invasiveness of tumour cells associated with a decrease in the number of filopodia, lamellipodia, and F-actin bundles [138]. A significant decrease in the level of Rho A (Ras homologue family member A) and depletion of mDia-2 (mammalian homologue of *Drosophila diaphanous* 2) from the cell periphery upon Hsp90 inhibition could also be observed. Both molecules are known to contribute to the generation of contractile forces and the formation of lamellipodia. Interestingly, Hsp90 inhibition was found to up-regulate the soluble form of actin (G-actin). Moreover, over-expression of αB-crystallin, known to be involved in actin dynamics, did not abrogate the effect of Hsp90 inhibition, indicating an enhanced interaction of Hsp90 with G-actin and αB-crystallin upon Hsp90 inhibition which might be responsible for the decreased actin treadmilling at the cell periphery.

4.2 HSP90s in Non-malignant Pathologies

Despite its impact in carcinogenesis, the involvement of HSP90s in various autoimmune diseases, including autoimmune bullous diseases and celiac disease, has been increasingly recognized. In patients with psoriasis, Hsp90α was significantly up-regulated in epidermal keratinocytes and mast cells of lesional skin [139]. Elevated plasma levels of Grp94 have been detected in patients with type 1 diabetes [140]. Altered circulating Hsp90 levels have also been reported in several pregnancy-related complications such as preeclampsia, gestational hypertension and fetal growth retardation. Pregnancy-related complications usually down-regulate Hsp90 in maternal whole peripheral blood [141]. In contrast, elevated Hsp90 levels could be detected in placental tissue of patients with mild preeclampsia, implying that Hsp90 up-regulation occurs just in case of long-term deteriorated conditions that facilitate prosecution of gestation by appropriate treatment approaches. An up-regulated Hsp90 expression could also be detected in human umbilical vein endothelial cells [142] and in umbilical cord blood red blood cells of preeclamptic subjects compared to normotensive subjects [143]. Notwithstanding this issue, the study by Zhang et al. [144] did not elicit any difference in Hsp90 levels in placentas from preeclamptic pregnancies compared to those from normotensive controls. However, additional investigations are required in order to establish

Hsp90 chaperones as robust biomarkers of these diseases.

Hsp90 is also critically implicated in the pathogenesis of infectious [145] and neuro-degenerative disorders such as Parkinson's disease (PD), Huntington's disease, Alzheimer's disease (AD), and frontotemporal dementia [146]. Hsp90 has been identified as the predominant HSP within filamentous inclusions in synucleinopathies, such as Lewy bodies dementia, PD and multiple system atrophy [147]. In this context, Hsp90 interacts with some intrinsically disordered proteins, including the microtubule-associated protein Tau. Hsp90 shows opposing effects on Tau turnover as it is able to promote both, Tau stabilization and degradation [148]. Mutant Tau is particularly susceptible to inhibition of Hsp90, making it a promising lead for therapeutic strategies in AD and other Tau-related disorders [148]. It is noteworthy that in AD patients reduced levels of Hsp90 in the diseased hippocampus, responsible for Tau accumulation, could be detected [149]. In patients with sporadic ALS, elevated levels of nitrated Hsp90 have been found in the insoluble fraction from human spinal cord tissues, underlining the crucial role of nitrative stress in aggregate formation in ALS [150]. Up-regulation of Hsp90 has recently been reported to associate with poor prognosis in patients with advanced myelodysplastic syndromes (MDS) in comparison to early MDS and bone marrow [151]. MDS are characterized by a high risk of progression to acute myeloid leukaemia. Recently, over-expression of Hsp90 has been associated with shorter survival and increased risk of progression into acute leukaemia in MDS [152]. These findings imply that assessment of the Hsp90 expression level in MDS might be predictive of patient response, allowing the selection of patients who might benefit from Hsp90 inhibition as a therapeutic approach.

An humoral autoimmune response to Hsp90 as determined by the appearance of anti-Hsp90 auto-antibodies has been reported in patients with dermatitis herpetiformis, [153], in sera of women with infertility [154], and in the cerebrospinal fluids from patients with Guillain-Barré syndrome [155]. Recently, the association between rheumatoid arthritis-associated interstitial lung disease and serum auto-antibodies recognizing citrullinated isoforms of Hsp90 was reported [156]. Evidence has emerged to demonstrate that serum antibodies directed against *P. gingivalis* HtpG are protective and thus predict health in peridontitis-susceptible patients and that response to periodontal therapy was more successful in subjects exhibiting higher levels of anti-*P. gingivalis* HtpG [157]. These findings might lead to early interventional therapy to prevent early-stage periodontal disease. Unfortunately, novel data on the potential of *P. gingivalis* HtpG as an effective diagnostic target and vaccine candidate are lacking up to date.

5. HSP90 INHIBITORS

Hsp90 functions as a crucial factor in tumourigenesis because it chaperones a wide spectrum of oncoproteins that are essential for the malignant transformation of cells. Therefore, targeting Hsp90 with chemical inhibitors would inactivate these oncogenic proteins and thus serves as a powerful anti-cancer strategy. Hsp90 has emerged in recent years as an important molecule in anti-tumour therapy, and several drug classes have been found to target its ATP-binding domain, culminating in inactivation of the chaperone. Alternatively, Hsp90 chaperone activity may be interrupted by small molecule binders, interfering with either the CTD or the NTD. Although Hsp90 function provides an attractive target for the treatment of cancer, the feasibility and efficacy of the inhibitor approach has just begun to be studied in clinical trials. Table 2 provides an overview of selected direct inhibitors of Hsp90. HSP90 inhibitors deplete Hsp90 client proteins in cancer cells without affecting their cellular counterparts in non-transformed cells [51,158]. The molecular basis for the selective anti-tumoural activity of HSP90 inhibitors appears to relate to the conformation of the Hsp90-inhibitor complex, as Hsp90 isolated from tumour cells has a 20 to 200 times higher binding affinity for the inhibitor than Hsp90 from non-transformed cells [51]. HSP90 inhibitors have the potential for use in single-agent or combinatorial therapies in order to supplement the hitherto existing conventional chemo-therapeutical approaches and molecularly targeted drugs.

There have been a number of particularly interesting and important findings relating to the role of Hsp90 in viral protein homeostasis. Hsp90 impacts the replication of numerous viruses, including DNA and RNA viruses as well as double-stranded RNA viruses [145]. Like many endogenous cellular proteins, various viral proteins have been characterized to depend on Hsp90 for their folding, assembly, maturation, and stability [145,159] thereby rendering Hsp90 a potential novel therapeutic target for the treatment of viral infections. The unrivalled

features of viral replication sensitize viruses to Hsp90 inhibition as demonstrated *in vitro* for Ebola virus, hepatitis C virus (HCV), herpes viruses, human immunodeficiency virus (HIV), influenza virus, paramyxoviruses, picornaviruses, La crosse virus, severe acquired respiratory syndrome (SARS) coronavirus, and vesicular stomatitis virus [145] as well as noroviruses [160], Chikungunya virus (CHIKV) [161]), and Kaposi sarcoma-associated herpes virus (KSHV) [162]). Meanwhile, the anti-viral capacity of HSP90 inhibitors has been confirmed *in vivo* for poliovirus [163], HCV [164], CHIKV [165], and KSHV-associated primary effusion lymphoma [166]. These findings encourage the use of HSP90 inhibitors for anti-viral therapeutic strategies in humans.

5.1 Natural HSP90 Inhibitors

The benzoquinone ansamycin geldanamycin (GDA), isolated from the broth of *Streptomyces hygroscopicus,* was about the first HSP90 inhibitor with promising anti-tumour and anti-viral properties in preclinical settings. GDA competes with ATP and binds to the NTD of Hsp90, thereby blocking its activity. GDA has been found to down-regulate Hsp90 client proteins including c-Raf, AKT, and Bcr-Abl, culminating in apoptosis induction [167]. GDA also induces degradation of several viral polymerases [168-170], DNA binding proteins such as ORF29p and Bag-3 [171] as well as of the polysomes translating protein A [172] and the non-structural protein 3 (NSP3) [173]. Since GDA shows several pharmacological limitations including poor solubility, limited *in vivo* stability and high hepatotoxicity in some of the human tumour models, analogues of GA, with similar biological behaviour but a better toxicity profile, were synthesized. Amongst them, 17-allylamino-17-demethoxygeldanamycin (17-AAG, tanespimycin) and 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG, alvespimycin) have entered clinical trials. These agents were found to destabilize and degrade numerous Hsp90 co-chaperones *in vivo* and showed promising anti-cancer properties in preclinical model systems [174]. In mantle cell lymphoma cell lines, 17-AAG induces cell cycle arrest and apoptosis by activating caspase-9 and depleting cyclin D1, AKT, and Bid [175]. Although 17-AAG elicits some encouraging clinical responses, it presents crucial drawbacks (*e.g.*, poor solubility, low bioavailability, liver toxicity and cumbersome formulation) that may limit its clinical application. Consequently, phase II studies in patients with metastatic breast cancer and metastatic melanoma were terminated because of apparent toxicity and lack of response [176,177]. 17-DMAG is an N,N-dimethylethylamino analogue of 17-AAG with improved solubility that entered phase I clinical trials where a tolerable toxicity was noted [178, 179]. In breast cancer, 17-DMAG was reported to mediate its anti-tumour effect through down-regulation of the receptor interacting protein 1 (Rip-1), thereby sensitizing breast cancer cells to TRAIL-induced apoptosis [180]. Models of murine medulloblastoma displayed a dependence on functional p53 to engage 17-DMAG-induced apoptosis [181]. In multiple myeloma, 17-DMAG attenuates STAT-3 and phospho-ERK levels that critically contribute to tumour cell survival in an IL-6R/STAT-3 and mitogen-activated protein kinase (MAPK)-dependent manner, respectively [182]. It should be pointed out that a phase II clinical trial of intravenous 17-DMAG for Her-2-positive breast cancer (ClinicalTrials.gov. Identifier: NCT00780000) was terminated for unknown reasons, underlining its limited clinical application. As outlined before, the Hsp90 clientele identified to date also include various viral proteins critically involved in viral protein homeostasis. Hsp90 inhibition by 17-AAG or 17-DMAG has been found to suppress viral replication by down-regulating a broad panel of viral Hsp90 clients, including NSP3 and poly(A)-binding protein [183], Bag-3 [171], the RNA-dependent RNA polymerase complex subunits PB1/-2 [184] as well as structural proteins such as VP1,VP2 and NS1/-2 [160].

IPI-504 (retaspimycin hydrochloride) is a new analogue of 17-AAG with improved water solubility suitable for parental administration. HSP90 inhibition by IPI-504 has been reported to induce apoptosis, block migration and invasion, and decrease epidermal growth factor receptor (EGFR) levels, MAPK and/or AKT activities, as well as secretion of vascular endothelial growth factor (VEGF) *in vitro* [185]. IPI-504 is the only first-generation Hsp90 inhibitor still under active development that entered phase II/III clinical trials. IPI-504 demonstrated an acceptable safety profile in phase II clinical trials conducted in patients with Her-2-positive breast cancer [186] and non-small cell lung cancer (NSCLC) [187]. However, a phase III trial of IPI-504 in patients with metastatic and/or unresectable gastro-intestinal stromal tumours (GIST) was stopped due to unexpected drug-related deaths [188].

Table 2. Direct inhibitors of HSP90

Name	Interaction site	General mechanism	References
Natural Hsp90 inhibitors			
Geldanamycin	NTD*	Down-regulation of c-Raf, AKT, Bcr-Abl; apoptosis induction	[167]
17-AAG (tanespimycin)	NTD	Down-regulation of Bid, c-Raf, cyclin D1, AKT, Bcr-Abl; apoptosis induction	[167,175]
17-DMAG (alvespimycin)	NTD	Down-regulation of ERK-1/2, Bcl-x, STAT-3, Rip-1; apoptosis induction	[181,182]
IPI-504 (retaspimycin hydrochloride, 17-AAG hydroquinone)	NTD	Apoptosis induction, blockage of cell migration and invasion, down-regulation of EGFR, decrease of MAPK and AKT activities, and VEGF secretion in glioma cells	[185,247,248]
Novobiocin	CTD*	Disruption of Hsp90 dimerization; destabilization of Hsp90 clients (Bcr-Abl, Her-2, mutant p53, Raf-1)	[189,194]
Radicicol	NTD	Depletion of Hsp90 clients (Bcr-Abl, Raf-1, Erb-B2, mutant p53)	[197]
(−)-Epigallocatechin-3-gallate (EGCG)	CTD	Down-regulation of Erb-B2, Raf-1, phospho-AKT	[212]
Curcumin	NTD	Depletion of Hsp90 clients (Bcr-Abl, STAT-5, CrkL EGFR, Raf-1, AKT, survivin), apoptosis induction	[203-206]
Taxol (paclitaxel)	CTD	Down-regulation of STAT-3/-5, Lyn, CrkL EGFR	[214-217]
Gambogic acid	NTD	Down-regulation of Hsp90 clients (Bcr-Abl, STAT-5, AKT, ERK-1/2, CrkL), apoptosis induction	[202]
5-Episinuleptolide acetate (5EPA)	unknown	Induction of apoptosis, down-regulation of c-Abl, NF-κB, AKT, Hsp90	[219]
Synthetic HSP90 inhibitors			
CCT-018159	NTD	Depletion of Erb-B2, Cdk-4, C-Raf, mutant B-Raf; up-regulation of Hsp70-1	[220]
VER-52296 (NVP-AUY922)	NTD	Blockage of tumour cell proliferation and tumour growth, apoptosis induction; degradation of HSP90, Bcr-Abl, Jak-2, Lyn and AKT; down-regulation of Cdk-4/6, AKT, survivin	[227-230]
VER-50589	NTD	Induction of Hsp70-1 and Hsp27; depletion of C-Raf, B-Raf, survivin, Prmt-5	[234]
KF58333	NTD	Depletion of Bcr-Abl, Raf-1, cell cycle-dependent kinases 4/-6; apoptosis induction	[221]
XL888	NTD	Apoptosis induction, down-regulation of Hsp90 clients (AKT, A-Raf, C-Raf, mutated N-Ras, IGF-1R, cyclin D1, PDGFRβ, MAP	[222-224]

Name	Interaction site	General mechanism	References
		kinase family member COT, Mcl-1), induction of Bim; degradation of Wee-1, Chk-1, Cdc-2; inhibition of MAPK, mTOR, c-jun NH_2 kinase (JNK)	
STA9090 (ganetespib)	NTD	Blockage of p23 coupling, down-regulation of *PDGFA*, *FGF2*, *ANG1*, *ANG2*, *TGFB1*, *VEGF*, *STAT3* and *HIF1A* mRNA	[225,226]
BIIB021 (CNF-2024)	NTD	Degradation of Her-2, AKT, Raf-1; induction of P-gp and Mrp-1; NF-κB depletion; up-regulation of MICA/B and ULBP2	[235-237]
SNX-25a	NTD	Cell cycle arrest, apoptosis induction, client protein degradation	[242]
Shepherdin	NTD	Antagonization of survivin interaction, down-regulation of client proteins (AKT, Cdk-4, Cdk-6)	[245]

**CTD: C-terminal domain; NTD: N-terminal domain*

The coumarin-related antibiotic novobiocin represents a further natural HSP90 inhibitor which interacts with the CTD of the chaperone, thereby disrupting its dimerization [189]. Novobiocin has been introduced into clinical use against *Staphylococcus aureus* infections, including multi-resistant MRSA strains. Novobiocin is also active against *Borrelia burgdorferi*, the causative agent of Lyme disease [190], Theiler's murine encephalomyelitis virus (TMEV) [191]), vaccinia virus [192], and KSHV [193]. Novobiocin has been shown to destabilize several Hsp90 client proteins, including Bcr-Abl, Her-2, mutant p53, and Raf-1 [194]. In Bcr-Abl-positive human leukemia cells, disruption of the Hsp90/Bcr-Abl complex by novobiocin induces cytosolic accumulation of cytochrome c and activation of caspase-9 and caspase-3, culminating in apoptosis induction [195]. Novel novobiocin derivatives such as KU135 proved to be more effective and selective HSP90 inhibitors [196].

Similar to GDA, the resorcyclic lactone radicicol, isolated from *Diheterospora chlamydosporia* and *Monosporium bonorden*, binds to the N-terminal ATP-binding site of Hsp90 and depletes Hsp90 client signalling molecules in cells, and thus inhibits signal transduction pathways [197]. Radicicol exerts anti-proliferative properties *in vitro* against KSHV [198], Ebola virus [199], paramyxoviruses and La Crosse bunyavirus [200] as well as against HCV *in vivo* [164]. However, radicicol lacks *in vivo* anti-cancer activity as it is converted to inactive metabolites *in vivo*. In order to resolve this limitation, oxime derivatives of radicicol such as KF58333 were synthesized (see chapter 5.2). The NTD is also the site of action of curcumin and gambogic acid (GA). GA, a main component of *Garcinia hanburyi*, directly interacts with the NTD of Hsp90 and induces apoptosis in tumour cells by down-regulating several Hsp90 client proteins, including Bcr-Abl, STAT-5, AKT, ERK-1/2, and CrkL [201,202]. GA has been approved by the Chinese FDA and entered phase II clinical trials. Curcumin, a potent anti-inflammatory and anti-tumourigenic agent isolated from *Curcuma longa*, interacts with the GDA binding pocket of Hsp90 [203]. It exhibits potent anti-proliferative properties in tumour cells by depleting numerous Hsp90 client proteins such as Bcr-Abl, STAT-5, CrkL EGFR, Raf-1, AKT, and the anti-apoptotic and mitotic regulator survivin [204-206]. Although studies on curcumin and its analogues have not yet fully overcome animal models, there is some clinical evidence of its beneficial anti-cancer support in humans. Currently, the addition of curcumin to FOLFOX-based chemotherapy has been shown to enhance killing in patient-derived colorectal liver metastases (CRLM) cultures by targeting cancer stem cells [207]. A phase I dose escalation study combining curcumin with first line FOLFOX chemotherapy in patients with CRLM has confirmed the safety and tolerability of this approach [207]. A randomised phase II study comparing participants receiving FOLFOX only with those receiving FOLFOX plus curcumin is currently recruiting. The potential use of curcumin as chemotherapeutic agent is rather optimistic and current studies are focused on improving its bioavailability and evaluating its efficaciousness in different malignancies. Interestingly, curcumin has emerged as a sophisticated preclinical agent in anti-viral strategies as it blocks virus replication by, *e.g.*, down-regulating the metabolic co-activator PGC-1α [208], suppressing the AKT/SREBP-1 pathway [209], inducing heme oxygenase-1 [210], and blocking the production of pro-inflammatory mediators (interleukins, TNF, NF-κB, PGHS-2) [211]. As curcumin also enhances the effect of conventional therapeutic drugs and minimizes their side effects [211], curcumin might be considered as a promising adjuvant in precise anti-viral therapies.

The ATP-binding site located at the Hsp90 C-terminus is known to allosterically modulate Hsp90's N-terminal ATPase activity. Thus, targeting the CTD represents a novel molecular strategy towards controlling Hsp90 chaperone activity. Several compounds have been shown to manifest Hsp90 inhibition by binding to the CTD, including (-)-epigallocatechin-3-gallate (EGCG) and taxol. The flavonoid EGCG, isolated from green tea *Camellia sinensis*, is the most abundant and powerful catechin in cancer prevention and treatment. It binds at or near to a C-terminal ATP-binding site of Hsp90, thereby preventing dimerization [212]. In human ovarian carcinoma cells, EGCG modifies the association of Hsp90 with several co-chaperones and decreases levels of several cancer-related Hsp90 client proteins, such as Erb-B2, Raf-1 and phospho-AKT [212]. Unfortunately, EGCG has poor drug-like properties because of its chemical and metabolic instability and low bioavailability. Recent approaches to develop novel more drug-like derivates of EGCG led to the identification of selected compounds that were more potent than EGCG [213]. Taxol, isolated from *Taxus baccata*, binds to the NTD of Hsp90 and inhibits its activity [214-216]. Interestingly, combined treatment of

taxol and the selective proteasome inhibitor bortezomib led to a marked decrease in Bcr-Abl protein levels and an inhibition of down-stream signalling pathways by depleting STAT-3/-5 as well as the Bcr-Abl-associated proteins CrkL and Lyn in tumour cells [217]. Combined treatment also activated several caspases and concomitant caspase-induced PARP cleavage culminating in apoptosis. It is of note that a prospective, single-armed, open label phase II study was conducted to evaluate the efficacy and safety of the combination of taxol (T)/cisplatin (P) with the humanized anti-EGFR monoclonal antibody nimotuzumab (N) as first-line treatment in advanced esophageal squamous cell cancer (ESCC). The TPN regimen has been found as being an effective combinatorial treatment as the first-line chemotherapy for patients with advanced ESCC, and appears more active than current standard regimens [218].

Recently, 5-episinuleptolide acetate (5EPA), a cytotoxic norcembranoidal diterpene from the Formosan soft coral *Sinularia sp*., has been shown to exhibit potent anti-proliferative activity against cancer cell lines. Additionally, the expression levels of Hsp90 protein and several client proteins were down-regulated in response to 5EPA [219]. However, no clinical data are available regarding the efficacy of this interesting compound in targeting Hsp90 in disease.

5.2 Synthetic HSP90 Inhibitors

In the past years, there has been a considerable increase in the discovery of HSP90 inhibitors, progressing from first-generation derivatives of natural products to second-generation fully synthetic small molecules. Structural drawbacks of GDA-based inhibitors have led to the development of several synthetically-derived HSP90 inhibitors, which are currently the focus of several clinical trials. CCT-018159 is a pyrazole analogue which binds to the NTD of Hsp90 similar to radicicol. The molecular signature of HSP90 inhibition comprises an up-regulation of Hsp70-1 protein and depletion of Erb-B2, Cdk-4, C-Raf, and mutant B-Raf [220]. Synthetic efforts that have been directed to identify radicicol derivatives with improved *in vivo* activity yielded its oxime derivate KF58333. KF58333 showed potent anti-tumour activities against human tumour xenograft models. Hsp90 client proteins such as Bcr-Abl and Raf-1 were depleted and apoptosis was induced in the tumour specimen treated with KF58333 [221]. XL888 represents a novel, orally available small molecule inhibitor of Hsp90α/β that binds to the N-terminal ATP-binding domain [222]. XL888 has been reported to overcome resistance to B-Raf inhibitors (vemurafenib and debrafenib) in preclinical models in different ways, including (i) blockage of the expression and/or functional activity of Hsp90 client proteins that are critical for growth and cell cycle re-entry (*e.g.*, AKT, A-Raf, C-Raf, mutated N-Ras, IGF-1R, cyclin D1, PDGFRβ, the MAPK family member COT); (ii) induction of the pro-apoptotic Bcl-2 interacting mediator of cell death (Bim); and (iii) down-regulation of Mcl-1 [223]. HSP90 inhibition has also been noted to degrade the crucial cell regulators Wee-1, Chk-1, and Cdc-2 and was associated with decreased MAPK, mTOR, and c-Jun N-terminal kinase (JNK) signalling in *NRAS*-mutant melanoma cells [224]. In an animal xenograft model of *NRAS*-mutant melanoma, XL888 treatment led to reduced tumour growth and apoptosis induction [224].

Several other small molecule HSP90 inhibitors have been reported in clinical settings including VER-52296 (NVP-AUY922), and STA9090 (ganetespib). STA9090, an unspecified new resorcinol-containing triazole compound, functions as a potent HSP90 modulator that has reached phase III clinical trials. STA9090 has been demonstrated as being broadly accepted in patients with solid tumours although dose-limiting toxicities were observed. The most common side effects comprise diarrhea, fatigue, nausea, and anorexia, which have been manageable with standard care. Preliminary signs of clinical activity have been monitored in NSCLC, breast cancer, gastric carcinoma, melanoma, and rectal carcinoma. In tumour samples from patients with rectal cancer, a down-regulation of *PDGFA*, *FGF2*, *ANG1*, *ANG2*, *TGFB1*, *VEGF*, *STAT3* and *HIF1A* mRNA was noted after STA9090 exposure [225]. In a different approach, STA9090 blocked the ability of Hsp90 to bind to biotinylated GDA and disrupted the association of Hsp90 with its co-chaperone, p23, more potently than 17-AAG [226]. The HSP90 ATPase inhibitor VER-52296 (NVP-AUY922) is an isoxazole analogue which exhibits potent anti-tumour activities *in vitro* and *in vivo* [227,228]. VER-52296 has been found to efficiently deplete numerous Hsp90 client proteins, including Bcr-Abl, Jak-2, Lyn, AKT, Cdk-4/6 and survivin *in vitro* [229,230]. Currently, VER-52296 is being evaluated in clinical phase I/II trials across a variety of malignancies where it exhibited common adverse effects such as diarrhea, nausea, and fatigue. Preliminary data from a

phase II clinical trial of VER-52296 monotherapy have shown partial responses in heavily pre-treated patients with advanced NSCLC [231]. Responses have also been reported in a phase IB/II trial of VER-52296 in combination with trastuzumab in Her-2-positive advanced breast cancer [232] as well as in a phase II trial of VER-52296 and the EGFR inhibitor erlotinib in patients with EGFR-mutant lung cancer and acquired resistance to EGFR tyrosine kinase inhibitors [233]. Optimization scaffolds yielded further isoxazole resorcinol inhibitors, *e.g.*, VER-50889. This isoxazole analogue exhibits a higher affinity and improved cellular uptake than the corresponding pyrazole analogues. Consistent with HSP90 inhibition, VER-50589 caused induction of Hsp70-1 and Hsp27 alongside depletion of client proteins, including C-Raf, B-Raf, survivin, and the protein arginine methyltransferase Prmt-5. Moreover, the compound caused cell cycle arrest and apoptosis as well as a 30% growth inhibition in human colon cancer xenografts [234].

Another group of synthetic HSP90 inhibitors comprises purine analogues, capable of competing with ADP/ATP for the ATP-binding site within Hsp90 and consequently inhibiting its chaperone functions. CNF-2024 (BIIB021) is a synthetic orally administrable purine-scaffold HSP90 inhibitor which competes with GDA for the ATP-binding pocket of Hsp90 and putatively down-regulates Her-2, AKT and Raf-1 *in vitro* and in several human tumour xenograft models *in vivo* [235]. CNF-2024 decreases Hodgkin lymphoma cell viability via NF-κB inhibition and up-regulates ligands for the activating NK cell receptor NKG2D (*e.g.,* MICA/B, ULBP2) on Hodgkin's lymphoma cells, thereby sensitizing the cells to NK cell-mediated killing [236]. Interestingly, CNF-2024 was found as being considerably more active than 17-AAG against adrenocortical carcinoma, both *in vitro* and *in vivo*, due to the expression of multidrug resistant proteins such as P-gp and Mrp-1 [237]. A clinical phase I dose escalation study of CNF-2024 administered orally in patients with advanced solid tumours revealed safety and tolerability [238]. Since a phase II clinical trial of CNF-2024 demonstrated feasibility and metabolic responses in >20% of patients with refractory GIST, one can assume that these data provide a strong foundation for future development of non-ansamycin HSP90 inhibitors in GIST [239].

Several other types of HSP90 inhibitors such as SNX-25a and shepherdin have been developed and successfully tested *in vitro* and *in vivo*. SNX-25a is a novel 2-aminobenzamide inhibitor of Hsp90 optimized by structure–activity relationship explorations for high Hsp90 affinity [240,241]. SNX-25a elicits a much higher efficacy than 17-AAG on growth inhibition of numerous cancer cells [242]. The mode of action of anti-tumour activity includes the induction of cell cycle arrest, apoptosis and Hsp90 client protein degradation. This superiority effect of SNX-25a warrants further confirmation in animal models, the more so as phase I clinical studies of the related compound SNX-5422 in patients with both, solid and hematological malagnancies have been disappointing [243,244]. Another interesting HSP90 inhibitor represents the cell-permeable peptidomimetic shepherdin which targets the N-terminal ATP-binding pocket of Hsp90, thereby antagonizing the interaction with survivin and down-regulating further Hsp90 clients (*e.g*., AKT, Cdk-4, Cdk-6) [245]. In this early study, shepherdin selectively induced death of tumour cells *in vitro* and inhibited human tumour growth in mice without toxicity [245]. More recently, the inhibition of survivin by shepherdin has been found to sensitize imatinib-resistant chronic myelogenous leukemia (CML) cells to different cytotoxic agents, implying that targeting Hsp90 with shepherdin might represent a promising therapeutic approach in patients with imatinib-resistant CML [246].

6. CONCLUSION

Hsp90 functions as an important hub in protein interaction networks involved in viability and protein homoeostasis. Hsp90 has emerged as a therapeutic target to treat a variety of disease states including neurodegenerative disorders, infectious and autoimmune diseases, pregnancy-related complications, myelodysplastic syndromes, and cancer. As outlined in this review, the rationale for using HSP90 inhibitors in cancer therapy is well established. Currently, HSP90 inhibitors are being evaluated in 52 clinical trials, of these VER-52296 (NVP-AUY922) and STA-9090 (ganetespib) are furthest in development. Clinical applications for Hsp90 modulation have topically focused on the treatment of cancer, and all clinically administered compounds act as competitive inhibitors of the N-terminal ATPase domain. All of these HSP90 modulators activate HSF-1 and induce the up-regulation of cytoprotective Hsp70 that might antagonize the pro-apoptotic properties of the inhibitors and limit their efficacy as anti-cancer agents. Thus, the clinical results

obtained up to date are insidious and should be perceived with great care. Consideration of the findings that are presented in this review raises the question of whether single-agent inhibitor therapy is the optimal strategy. Nowadays much interest has been attracted to the use of HSP90 inhibitors in combination with cutting-edge targeted therapies. Preclinical data from different cancer type models imply that HSP90 inhibitors have the capacity to improve the outcome of other anti-cancer approaches, including chemotherapy, kinase inhibitors, and radiation therapy, and to potentially overcome drug resistance [249]. In this regard, the combination of the HSP90 inhibitor SNX-5422 and trastuzumab (herceptin), a monoclonal antibody that blocks the Her-2 receptor, has been shown to synergistically regress tumour growth in a xenograft model of human breast cancer [250]. On the understanding that the synergistic effects in tumour regression observed in animal studies after combinatorial administration of HSP90 inhibitors and potent anti-cancer drugs hold true in human trials, targeted therapies are about to come. A first hint is given by a phase II trial, in which the combinatorial administration of 17-AAG (tanespimycin) plus trastuzumab has significant anti-cancer activity in patients with Her-2-positive, metastatic breast cancer previously progressing on trastuzumab [251]. Referring to the information above, a combinatorial administration of HSP90 inhibitors and HSF-1 inhibitors might be a rationale to overcome the negative effects of HSP90 inhibitors. Notwithstanding this issue, HSP90 inhibitors have emerged as promising radiosensitizers. In a recent study by the group of Gabriele Multhoff, the HSR inhibitor NZ28, either alone or in combination with the HSP90 inhibitor VER-52296 (NVP-AUY922), was investigated for radiosensitizing effects of radioresistant tumour cells. The results clearly demonstrate that a dual targeting of Hsp70 and Hsp90 with NZ28 and VER-52296 (NVP-AUY922) potentiates the radiation response of tumour cells that are otherwise resistant to ionizing radiation [252]. A simultaneous inhibition of Hsp90 and HSF1/Hsp70 combined with radiotherapy can thus be considered as being a promising anti-cancer strategy. Our own findings favor a therapeutic approach which takes advantage of the radiosensitizing effects of certain phytochemicals such as curcumin or EGCG. These phytochemicals sensitize tumour cells to radio-/chemotherapy by inhibiting pathways critically involved in treatment resistance. Both agents have been identified to bind to Hsp70 and Hsp90 and consequently modulate their activities. We and others have shown that EGCG and curcumin inhibt activation of NF-κB in tumour cells [253-255] which acts as a central linker between inflammation, malignant transformation, and radioresistance. These flavonoids and other plant-derived polyphenols have been studied intensively for their chemopreventive potential and have been found as being pharmacological safe. The use of either EGCG or curcumin in combination with radio-/chemotherapy might represent an ambitious HSP inhibition scenario in order to sensitize tumours to ionizing radiation and chemotherapeutic drugs. Currently, several phase I/II clinical trials are reported underway to test the feasibility, safety and efficacy of EGCG in sensitizing cancer patients to radio-/chemotherapy. It is worth mentioning that EGCG has been identified to potentiate efficacy of radiotherapy in breast cancer patients [256]. Such a combination strategy might have future clinical implications with respect to the development of novel approaches as an adjuvant therapy in cancer.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Ritossa F. A new puffing pattern induced by temperature shock and DNP in *Drosophila*. Experientia. 1962;18571-73.
2. Csermely P, Schnaider T, Soti C, Prohaszka Z, Nardai G. The 90-kDa molecular chaperone family: Structure, function, and clinical applications. A comprehensive review. Pharmacol Ther. 1998;79(2):129-68.
3. Felts SJ, Owen BA, Nguyen P, Trepel J, Donner DB, Toft DO. The hsp90-related protein TRAP1 is a mitochondrial protein with distinct functional properties. J Biol Chem. 2000;275(5):3305-12.
4. Krishna P, Gloor G. The Hsp90 family of proteins in *Arabidopsis thaliana*. Cell Stress Chaperones. 2001;6(3):238-46.

5. Hightower LE, Guidon PT, Jr. Selective release from cultured mammalian cells of heat-shock (stress) proteins that resemble glia-axon transfer proteins. J Cell Physiol. 1989;138(2):257-66.
6. Clayton A, Turkes A, Navabi H, Mason MD, Tabi Z. Induction of heat shock proteins in B-cell exosomes. J Cell Sci. 2005;118(Pt 16):3631-38.
7. Basu S, Binder RJ, Suto R, Anderson KM, Srivastava PK. Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-kappa B pathway. Int Immunol. 2000;12(11):1539-46.
8. Hegmans JP, Bard MP, Hemmes A, Luider TM, Kleijmeer MJ, Prins JB, et al. Proteomic analysis of exosomes secreted by human mesothelioma cells. Am J Pathol. 2004;164(5):1807-15.
9. Nickel W. The mystery of nonclassical protein secretion. A current view on cargo proteins and potential export routes. Eur J Biochem. 2003;270(10):2109-19.
10. Chen B, Zhong D, Monteiro A. Comparative genomics and evolution of the HSP90 family of genes across all kingdoms of organisms. BMC Genomics. 2006;7156.
11. Stechmann A, Cavalier-Smith T. Evolutionary origins of Hsp90 chaperones and a deep paralogy in their bacterial ancestors. J Eukaryot Microbiol. 2004; 51(3):364-73.
12. Chen B, Piel WH, Gui L, Bruford E, Monteiro A. The HSP90 family of genes in the human genome: Insights into their divergence and evolution. Genomics. 2005;86(6):627-37.
13. Krone PH, Sass JB. HSP 90 alpha and HSP 90 beta genes are present in the zebrafish and are differentially regulated in developing embryos. Biochem Biophys Res Commun. 1994;204(2):746-52.
14. Han G, Ye M, Zhou H, Jiang X, Feng S, Jiang X et al. Large-scale phospho-proteome analysis of human liver tissue by enrichment and fractionation of phospho-peptides with strong anion exchange chromatography. Proteomics. 2008;8(7): 1346-61.
15. Gupta RS. Phylogenetic analysis of the 90 kD heat shock family of protein sequences and an examination of the relationship among animals, plants, and fungi species. Mol Biol Evol. 1995;12(6):1063-73.
16. Nicchitta CV. Biochemical, cell biological and immunological issues surrounding the endoplasmic reticulum chaperone GRP94/ gp96. Curr Opin Immunol. 1998;10(1):103-9.
17. Tamura Y, Peng P, Liu K, Daou M, Srivastava PK. Immunotherapy of tumors with autologous tumor-derived heat shock protein preparations. Science. 1997; 278(5335):117-20.
18. Yoshida S, Tsutsumi S, Muhlebach G, Sourbier C, Lee MJ, Lee S, et al. Molecular chaperone TRAP1 regulates a metabolic switch between mitochondrial respiration and aerobic glycolysis. Proc Natl Acad Sci U S A. 2013;110(17):E1604-E1612.
19. Li J, Soroka J, Buchner J. The Hsp90 chaperone machinery: Conformational dynamics and regulation by co-chaperones. Biochim Biophys Acta. 2012; 1823(3):624-35.
20. Echeverria PC, Bernthaler A, Dupuis P, Mayer B, Picard D. An interaction network predicted from public data as a discovery tool: application to the Hsp90 molecular chaperone machine. PLoS ONE. 2011; 6(10):e26044.
21. Samant RS, Clarke PA, Workman P. The expanding proteome of the molecular chaperone HSP90. Cell Cycle. 2012;11(7): 1301-8.
22. Taipale M, Krykbaeva I, Koeva M, Kayatekin C, Westover KD, Karras GI et al. Quantitative analysis of HSP90-client interactions reveals principles of substrate recognition. Cell. 2012;150(5):987-1001.
23. Singh SD, Robbins N, Zaas AK, Schell WA, Perfect JR, Cowen LE. Hsp90 governs echinocandin resistance in the pathogenic yeast *Candida albicans* via calcineurin. PLoS Pathog. 2009;5(7): e1000532.
24. Minami Y, Kimura Y, Kawasaki H, Suzuki K, Yahara I. The carboxy-terminal region of mammalian HSP90 is required for its dimerization and function in vivo. Mol Cell Biol. 1994;14(2):1459-64.
25. Nemoto T, Sato N. Oligomeric forms of the 90-kDa heat shock protein. Biochem J. 1998;330(Pt 2):989-95.
26. Yonehara M, Minami Y, Kawata Y, Nagai J, Yahara I. Heat-induced chaperone activity of HSP90. J Biol Chem. 1996;271(5):2641-45.
27. Nemoto TK, Ono T, Tanaka K. Substrate-binding characteristics of proteins in the 90

kDa heat shock protein family. Biochem J. 2001;354(Pt 3):663-70.
28. Ali MM, Roe SM, Vaughan CK, Meyer P, Panaretou B, Piper PW, et al. Crystal structure of an Hsp90-nucleotide-p23/Sba1 closed chaperone complex. Nature. 2006; 440(7087):1013-17.
29. Munro S, Pelham HR. A C-terminal signal prevents secretion of luminal ER proteins. Cell. 1987;48(5):899-907.
30. Whitesell L, Shifrin SD, Schwab G, Neckers LM. Benzoquinonoid ansamycins possess selective tumoricidal activity unrelated to src kinase inhibition. Cancer Res. 1992;52(7):1721-28.
31. Roe SM, Prodromou C, O'Brien R, Ladbury JE, Piper PW, Pearl LH. Structural basis for inhibition of the Hsp90 molecular chaperone by the antitumor antibiotics radicicol and geldanamycin. J Med Chem. 1999;42(2):260-266.
32. Pearl LH, Prodromou C. Structure and in vivo function of Hsp90. Curr Opin Struct Biol. 2000;10(1):46-51.
33. Dixit A, Verkhivker GM. Probing molecular mechanisms of the Hsp90 chaperone: Biophysical modeling identifies key regulators of functional dynamics. PLoS ONE. 2012;7(5):e37605.
34. Zuehlke A, Johnson JL. Hsp90 and co-chaperones twist the functions of diverse client proteins. Biopolymers. 2010;93(3): 211-17.
35. Shiau AK, Harris SF, Southworth DR, Agard DA. Structural analysis of *E. coli* hsp90 reveals dramatic nucleotide-dependent conformational rearrange-ments. Cell. 2006;127(2):329-40.
36. Dollins DE, Warren JJ, Immormino RM, Gewirth DT. Structures of GRP94-nucleotide complexes reveal mechanistic differences between the hsp90 chaperones. Mol Cell. 2007;28(1):41-56.
37. Prodromou C, Panaretou B, Chohan S, Siligardi G, O'Brien R, Ladbury JE et al. The ATPase cycle of Hsp90 drives a molecular 'clamp' via transient dimerization of the N-terminal domains. EMBO J. 2000;19(16):4383-92.
38. Blacklock K, Verkhivker GM. Allosteric regulation of the Hsp90 dynamics and stability by client recruiter cochaperones: protein structure network modeling. PLoS ONE. 2014;9(1):e86547.
39. Krukenberg KA, Forster F, Rice LM, Sali A, Agard DA. Multiple conformations of *E. coli* Hsp90 in solution: insights into the conformational dynamics of Hsp90. Structure. 2008;16(5):755-65.
40. Cheng CF, Fan J, Zhao Z, Woodley DT, Li W. Secreted heat shock protein-90a: a more effective and safer target for anti-cancer drugs? Curr Signal Transduct Ther. 2010;5(2):121-27.
41. Makhnevych T, Houry WA. The role of Hsp90 in protein complex assembly. Biochim Biophys Acta. 2012;1823(3):674-82.
42. Caplan AJ, Jackson S, Smith D. Hsp90 reaches new heights. Conference on the Hsp90 chaperone machine. EMBO Rep. 2003;4(2):126-30.
43. Wegele H, Müller L, Buchner J. Hsp70 and Hsp90 - A relay team for protein folding. Rev Physiol Biochem Pharmacol. 2004; 1511-44.
44. Jackson SE, Queitsch C, Toft D. Hsp90: From structure to phenotype. Nat Struct Mol Biol. 2004;11(12):1152-55.
45. Pockley AG, Henderson B, Multhoff G. Extracellular cell stress proteins as biomarkers of human disease. Biochem Soc Trans. 2014;42(6):1744-51.
46. Taipale M, Jarosz DF, Lindquist S. HSP90 at the hub of protein homeostasis: Emerging mechanistic insights. Nat Rev Mol Cell Biol. 2010;11(7):515-28.
47. Zhao R, Houry WA. Molecular interaction network of the Hsp90 chaperone system. Adv Exp Med Biol. 2007;59427-36.
48. Zhao R, Davey M, Hsu YC, Kaplanek P, Tong A, Parsons AB, et al. Navigating the chaperone network: An integrative map of physical and genetic interactions mediated by the hsp90 chaperone. Cell. 2005; 120(5):715-27.
49. Calderwood SK, Khaleque MA, Sawyer DB, Ciocca DR. Heat shock proteins in cancer: Chaperones of tumorigenesis. Trends Biochem Sci. 2006;31(3):164-72.
50. Xu W, Neckers L. Targeting the molecular chaperone heat shock protein 90 provides a multifaceted effect on diverse cell signaling pathways of cancer cells. Clin Cancer Res. 2007;13(6):1625-29.
51. Kamal A, Thao L, Sensintaffar J, Zhang L, Boehm MF, Fritz LC, et al. A high-affinity conformation of Hsp90 confers tumour selectivity on Hsp90 inhibitors. Nature. 2003;425(6956):407-10.
52. Chen Z, Sasaki T, Tan X, Carretero J, Shimamura T, Li D, et al. Inhibition of ALK, PI3K/MEK, and HSP90 in murine lung adenocarcinoma induced by EML4-ALK

fusion oncogene. Cancer Res. 2010; 70(23):9827-36.

53. Cano LQ, Lavery DN, Bevan CL. Mini-review: Foldosome regulation of androgen receptor action in prostate cancer. Mol Cell Endocrinol. 2013;369(1-2):52-62.
54. Eckl JM, Richter K. Functions of the Hsp90 chaperone system: Lifting client proteins to new heights. Int J Biochem Mol Biol. 2013;4(4):157-65.
55. Hildenbrand ZL, Molugu SK, Herrera N, Ramirez C, Xiao C, Bernal RA. Hsp90 can accommodate the simultaneous binding of the FKBP52 and HOP proteins. Oncotarget. 2011;2(1-2):43-58.
56. Pratt WB, Toft DO. Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. Exp Biol Med (Maywood). 2003;228(2): 111-33.
57. Hernandez MP, Sullivan WP, Toft DO. The assembly and intermolecular properties of the hsp70-Hop-hsp90 molecular chaperone complex. J Biol Chem. 2002;277(41): 38294-304.
58. Gaiser AM, Brandt F, Richter K. The non-canonical Hop protein from *Caenorhabditis elegans* exerts essential functions and forms binary complexes with either Hsc70 or Hsp90. J Mol Biol. 2009;391(3):621-34.
59. McLaughlin SH, Smith HW, Jackson SE. Stimulation of the weak ATPase activity of human hsp90 by a client protein. J Mol Biol. 2002;315(4):787-98.
60. Ebong IO, Morgner N, Zhou M, Saraiva MA, Daturpalli S, Jackson SE, et al. Heterogeneity and dynamics in the assembly of the heat shock protein 90 chaperone complexes. Proc Natl Acad Sci U S A. 2011;108(44):17939-44.
61. Li J, Richter K, Buchner J. Mixed Hsp90-cochaperone complexes are important for the progression of the reaction cycle. Nat Struct Mol Biol. 2011;18(1):61-66.
62. Morishima Y, Kanelakis KC, Murphy PJ, Lowe ER, Jenkins GJ, Osawa Y, et al. The hsp90 cochaperone p23 is the limiting component of the multiprotein hsp90/hsp70-based chaperone system in vivo where it acts to stabilize the client protein: hsp90 complex. J Biol Chem. 2003;278(49):48754-63.
63. Harst A, Lin H, Obermann WM. Aha1 competes with Hop, p50 and p23 for binding to the molecular chaperone Hsp90 and contributes to kinase and hormone receptor activation. Biochem J. 2005; 387(Pt 3):789-96.
64. Pratt WB, Toft DO. Steroid receptor interactions with heat shock protein and immunophilin chaperones. Endocr Rev. 1997;18(3):306-60.
65. Simons SS Jr, Sistare FD, Chakraborti PK. Steroid binding activity is retained in a 16-kDa fragment of the steroid binding domain of rat glucocorticoid receptors. J Biol Chem. 1989;264(24):14493-97.
66. Bledsoe RK, Montana VG, Stanley TB, Delves CJ, Apolito CJ, McKee DD et al. Crystal structure of the glucocorticoid receptor ligand binding domain reveals a novel mode of receptor dimerization and coactivator recognition. Cell. 2002;110(1): 93-105.
67. Galigniana MD, Echeverria PC, Erlejman AG, Piwien-Pilipuk G. Role of molecular chaperones and TPR-domain proteins in the cytoplasmic transport of steroid receptors and their passage through the nuclear pore. Nucleus. 2010;1(4):299-308.
68. Davies TH, Ning YM, Sanchez ER. A new first step in activation of steroid receptors: hormone-induced switching of FKBP51 and FKBP52 immunophilins. J Biol Chem. 2002;277(7):4597-600.
69. Li W, Tsen F, Sahu D, Bhatia A, Chen M, Multhoff G, et al. Extracellular Hsp90 (eHsp90) as the actual target in clinical trials: intentionally or unintentionally. Int Rev Cell Mol Biol. 2013;303203-35.
70. Yu X, Harris SL, Levine AJ. The regulation of exosome secretion: A novel function of the p53 protein. Cancer Res. 2006;66(9): 4795-801.
71. Sarkar AA, Zohn IE. Hectd1 regulates intracellular localization and secretion of Hsp90 to control cellular behavior of the cranial mesenchyme. J Cell Biol. 2012; 196(6):789-800.
72. Li W, Li Y, Guan S, Fan J, Cheng CF, Bright AM, et al. Extracellular heat shock protein-90alpha: linking hypoxia to skin cell motility and wound healing. EMBO J. 2007;26(5):1221-33.
73. Woodley DT, Fan J, Cheng CF, Li Y, Chen M, Bu G, et al. Participation of the lipoprotein receptor LRP1 in hypoxia-HSP90alpha autocrine signaling to promote keratinocyte migration. J Cell Sci. 2009;122(Pt 10):1495-98.
74. Arnold-Schild D, Hanau D, Spehner D, Schmid C, Rammensee HG, de la SH, et al. Cutting edge: Receptor-mediated

endocytosis of heat shock proteins by professional antigen-presenting cells. J Immunol. 1999;162(7):3757-60.
75. Singh-Jasuja H, Toes RE, Spee P, Munz C, Hilf N, Schoenberger SP et al. Cross-presentation of glycoprotein 96-associated antigens on major histocompatibility complex class I molecules requires receptor-mediated endocytosis. J Exp Med. 2000;191(11):1965-74.
76. Srivastava PK, Menoret A, Basu S, Binder RJ, McQuade KL. Heat shock proteins come of age: Primitive functions acquire new roles in an adaptive world. Immunity. 1998;8(6):657-65.
77. Binder RJ, Vatner R, Srivastava P. The heat-shock protein receptors: Some answers and more questions. Tissue Antigens. 2004;64(4):442-51.
78. Henderson B, Kaiser F. Do reciprocal interactions between cell stress proteins and cytokines create a new intra-/extra-cellular signalling nexus? Cell Stress Chaperones. 2013;18(6):685-701.
79. Zhu H, Fang X, Zhang D, Wu W, Shao M, Wang L et al. Membrane-bound heat shock proteins facilitate the uptake of dying cells and cross-presentation of cellular antigen. Apoptosis. 2016;21(1):96-109.
80. Zeng Y, Chen X, Larmonier N, Larmonier C, Li G, Sepassi M et al. Natural killer cells play a key role in the antitumor immunity generated by chaperone-rich cell lysate vaccination. Int J Cancer. 2006;119(11): 2624-31.
81. Broemer M, Krappmann D, Scheidereit C. Requirement of Hsp90 activity for IkappaB kinase (IKK) biosynthesis and for constitutive and inducible IKK and NF-kappaB activation. Oncogene. 2004; 23(31):5378-86.
82. Shimp SK, III, Parson CD, Regna NL, Thomas AN, Chafin CB, Reilly CM, et al. HSP90 inhibition by 17-DMAG reduces inflammation in J774 macrophages through suppression of Akt and nuclear factor-kappaB pathways. Inflamm Res. 2012;61(5):521-33.
83. Dowling P, Walsh N, Clynes M. Membrane and membrane-associated proteins involved in the aggressive phenotype displayed by highly invasive cancer cells. Proteomics. 2008;8(19):4054-65.
84. Cheng CF, Fan J, Fedesco M, Guan S, Li Y, Bandyopadhyay B, et al. Transforming growth factor alpha (TGFalpha)-stimulated secretion of HSP90alpha: Using the receptor LRP-1/CD91 to promote human skin cell migration against a TGFbeta-rich environment during wound healing. Mol Cell Biol. 2008;28(10):3344-58.
85. Eustace BK, Sakurai T, Stewart JK, Yimlamai D, Unger C, Zehetmeier C, et al. Functional proteomic screens reveal an essential extracellular role for hsp90 alpha in cancer cell invasiveness. Nat Cell Biol. 2004;6(6):507-14.
86. Stellas D, El HA, Patsavoudi E. Monoclonal antibody 4C5 prevents activation of MMP2 and MMP9 by disrupting their interaction with extra-cellular HSP90 and inhibits formation of metastatic breast cancer cell deposits. BMC Cell Biol. 2010;1151.
87. Sidera K, Gaitanou M, Stellas D, Matsas R, Patsavoudi E. A critical role for HSP90 in cancer cell invasion involves interaction with the extracellular domain of HER-2. J Biol Chem. 2008;283(4):2031-41.
88. Shamovsky I, Nudler E. New insights into the mechanism of heat shock response activation. Cell Mol Life Sci. 2008;65(6): 855-61.
89. Craig EA. The stress response: changes in eukaryotic gene expression in response to environmental stress. Science. 1985; 230(4727):800-801.
90. Calderwood SK, Gong J. Molecular chaperones in mammary cancer growth and breast tumor therapy. J Cell Biochem. 2012;113(4):1096-103.
91. Hayashida N, Fujimoto M, Tan K, Prakasam R, Shinkawa T, Li L, et al. Heat shock factor 1 ameliorates proteotoxicity in cooperation with the transcription factor NFAT. EMBO J. 2010;29(20):3459-69.
92. Pirkkala L, Nykanen P, Sistonen L. Roles of the heat shock transcription factors in regulation of the heat shock response and beyond. FASEB J. 2001;15(7):1118-31.
93. Akerfelt M, Morimoto RI, Sistonen L. Heat shock factors: integrators of cell stress, development and lifespan. Nat Rev Mol Cell Biol. 2010;11(8):545-55.
94. Hietakangas V, Ahlskog JK, Jakobsson AM, Hellesuo M, Sahlberg NM, Holmberg CI et al. Phosphorylation of serine 303 is a prerequisite for the stress-inducible SUMO modification of heat shock factor 1. Mol Cell Biol. 2003;23(8):2953-68.
95. Westwood JT, Clos J, Wu C. Stress-induced oligomerization and chromosomal relocalization of heat-shock factor. Nature. 1991;353(6347):822-27.

96. Chou SD, Prince T, Gong J, Calderwood SK. mTOR is essential for the proteotoxic stress response, HSF1 activation and heat shock protein synthesis. PLoS ONE. 2012;7(6):e39679.
97. Shi Y, Mosser DD, Morimoto RI. Molecular chaperones as HSF1-specific transcriptional repressors. Genes Dev. 1998;12(5): 654-66.
98. Westerheide SD, Anckar J, Stevens SM, Jr., Sistonen L, Morimoto RI. Stress-inducible regulation of heat shock factor 1 by the deacetylase SIRT1. Science. 2009;323(5917):1063-66.
99. Stephanou A, Isenberg DA, Akira S, Kishimoto T, Latchman DS. The nuclear factor interleukin-6 (NF-IL6) and signal transducer and activator of transcription-3 (STAT-3) signalling pathways co-operate to mediate the activation of the hsp90beta gene by interleukin-6 but have opposite effects on its inducibility by heat shock. Biochem J. 1998;330(Pt 1):189-95.
100. Akira S, Nishio Y, Inoue M, Wang XJ, Wei S, Matsusaka T, et al. Molecular cloning of APRF, a novel IFN-stimulated gene factor 3 p91-related transcription factor involved in the gp130-mediated signaling pathway. Cell. 1994;77(1):63-71.
101. Stephanou A, Isenberg DA, Nakajima K, Latchman DS. Signal transducer and activator of transcription-1 and heat shock factor-1 interact and activate the transcription of the Hsp-70 and Hsp-90beta gene promoters. J Biol Chem. 1999; 274(3):1723-28.
102. Ammirante M, Rosati A, Gentilella A, Festa M, Petrella A, Marzullo L, et al. The activity of hsp90 alpha promoter is regulated by NF-kappa B transcription factors. Oncogene. 2008;27(8):1175-78.
103. Rappa F, Farina F, Zummo G, David S, Campanella C, Carini F, et al. HSP-molecular chaperones in cancer biogenesis and tumor therapy: An overview. Anticancer Res. 2012;32(12):5139-50.
104. Tu J, Liao J, Luk AC, Tang NL, Chan WY, Lee TL. MicroRNAs mediated targeting on the Yin-yang dynamics of DNA methylation in disease and development. Int J Biochem Cell Biol. 2015;67115-20.
105. Li G, Cai M, Fu D, Chen K, Sun M, Cai Z et al. Heat shock protein 90B1 plays an oncogenic role and is a target of microRNA-223 in human osteosarcoma. Cell Physiol Biochem. 2012;30(6):1481-90.
106. Kariya A, Furusawa Y, Yunoki T, Kondo T, Tabuchi Y. A microRNA-27a mimic sensitizes human oral squamous cell carcinoma HSC-4 cells to hyperthermia through downregulation of Hsp110 and Hsp90. Int J Mol Med. 2014;34(1):334-40.
107. Matkovich SJ, Hu Y, Eschenbacher WH, Dorn LE, Dorn GW. Direct and indirect involvement of microRNA-499 in clinical and experimental cardiomyopathy. Circ Res. 2012;111(5):521-31.
108. Bi R, Bao C, Jiang L, Liu H, Yang Y, Mei J et al. MicroRNA-27b plays a role in pulmonary arterial hypertension by modulating peroxisome proliferator-activated receptor gamma dependent Hsp90-eNOS signaling and nitric oxide production. Biochem Biophys Res Commun. 2015;460(2):469-75.
109. O'Brien K, Lowry MC, Corcoran C, Martinez VG, Daly M, Rani S, et al. miR-134 in extracellular vesicles reduces triple-negative breast cancer aggression and increases drug sensitivity. Oncotarget. 2015;6(32):32774-89.
110. Guo Y, Liu J, Elfenbein SJ, Ma Y, Zhong M, Qiu C, et al. Characterization of the mammalian miRNA turnover landscape. Nucleic Acids Res. 2015;43(4):2326-41.
111. Mollapour M, Neckers L. Post-translational modifications of Hsp90 and their contributions to chaperone regulation. Biochim Biophys Acta. 2012;1823(3):648-55.
112. Mollapour M, Tsutsumi S, Truman AW, Xu W, Vaughan CK, Beebe K et al. Threonine 22 phosphorylation attenuates Hsp90 interaction with cochaperones and affects its chaperone activity. Mol Cell. 2011; 41(6):672-81.
113. Mimnaugh EG, Worland PJ, Whitesell L, Neckers LM. Possible role for serine/threonine phosphorylation in the regulation of the heteroprotein complex between the hsp90 stress protein and the pp60v-src tyrosine kinase. J Biol Chem. 1995;270(48):28654-59.
114. Lei H, Venkatakrishnan A, Yu S, Kazlauskas A. Protein kinase A-dependent translocation of Hsp90 alpha impairs endothelial nitric-oxide synthase activity in high glucose and diabetes. J Biol Chem. 2007;282(13):9364-71.
115. Wang X, Song X, Zhuo W, Fu Y, Shi H, Liang Y, et al. The regulatory mechanism of Hsp90alpha secretion and its function in

tumor malignancy. Proc Natl Acad Sci U S A. 2009;106(50):21288-93.
116. Scroggins BT, Robzyk K, Wang D, Marcu MG, Tsutsumi S, Beebe K, et al. An acetylation site in the middle domain of Hsp90 regulates chaperone function. Mol Cell. 2007;25(1):151-59.
117. Murphy PJ, Morishima Y, Kovacs JJ, Yao TP, Pratt WB. Regulation of the dynamics of hsp90 action on the glucocorticoid receptor by acetylation/deacetylation of the chaperone. J Biol Chem. 2005;280(40): 33792-99.
118. Kovacs JJ, Murphy PJ, Gaillard S, Zhao X, Wu JT, Nicchitta CV, et al. HDAC6 regulates Hsp90 acetylation and chaperone-dependent activation of glucocorticoid receptor. Mol Cell. 2005; 18(5):601-7.
119. Park JH, Kim SH, Choi MC, Lee J, Oh DY, Im SA, et al. Class II histone deacetylases play pivotal roles in heat shock protein 90-mediated proteasomal degradation of vascular endothelial growth factor receptors. Biochem Biophys Res Commun. 2008;368(2):318-22.
120. Lee SM, Bae JH, Kim MJ, Lee HS, Lee MK, Chung BS, et al. Bcr-Abl-independent imatinib-resistant K562 cells show aberrant protein acetylation and increased sensitivity to histone deacetylase inhibitors. J Pharmacol Exp Ther. 2007;322(3):1084-92.
121. Retzlaff M, Stahl M, Eberl HC, Lagleder S, Beck J, Kessler H, et al. Hsp90 is regulated by a switch point in the C-terminal domain. EMBO Rep. 2009;10(10): 1147-53.
122. Piwien-Pilipuk G, Ayala A, Machado A, Galigniana MD. Impairment of mineralocorticoid receptor (MR)-dependent biological response by oxidative stress and aging: Correlation with post-translational modification of MR and decreased ADP-ribosylatable level of elongating factor 2 in kidney cells. J Biol Chem. 2002;277(14): 11896-903.
123. Abu-Farha M, Lanouette S, Elisma F, Tremblay V, Butson J, Figeys D, et al. Proteomic analyses of the SMYD family interactomes identify HSP90 as a novel target for SMYD2. J Mol Cell Biol. 2011; 3(5):301-8.
124. Hamamoto R, Toyokawa G, Nakakido M, Ueda K, Nakamura Y. SMYD2-dependent HSP90 methylation promotes cancer cell proliferation by regulating the chaperone complex formation. Cancer Lett. 2014; 351(1):126-33.
125. Franco MC, Ye Y, Refakis CA, Feldman JL, Stokes AL, Basso M, et al. Nitration of Hsp90 induces cell death. Proc Natl Acad Sci U S A. 2013;110(12):E1102-E1111.
126. Mollapour M, Bourboulia D, Beebe K, Woodford MR, Polier S, Hoang A, et al. Asymmetric Hsp90 N domain SUMOylation recruits Aha1 and ATP-competitive inhibitors. Mol Cell. 2014;53(2):317-29.
127. Kundrat L, Regan L. Identification of residues on Hsp70 and Hsp90 ubiquitinated by the cochaperone CHIP. J Mol Biol. 2010;395(3):587-94.
128. Ciocca DR, Calderwood SK. Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. Cell Stress Chaperones. 2005;10(2):86-103.
129. Sevin M, Girodon F, Garrido C, de TA. HSP90 and HSP70: Implication in inflammation processes and therapeutic approaches for myeloproliferative neoplasms. Mediators Inflamm. 2015; 2015:970242.
130. McCarthy MM, Pick E, Kluger Y, Gould-Rothberg B, Lazova R, Camp RL, et al. HSP90 as a marker of progression in melanoma. Ann Oncol. 2008;19(3):590-594.
131. Lim SO, Park SG, Yoo JH, Park YM, Kim HJ, Jang KT et al. Expression of heat shock proteins (HSP27, HSP60, HSP70, HSP90, GRP78, GRP94) in hepatitis B virus-related hepatocellular carcinomas and dysplastic nodules. World J Gastroenterol. 2005;11(14):2072-79.
132. Lebret T, Watson RW, Molinie V, O'Neill A, Gabriel C, Fitzpatrick JM, et al. Heat shock proteins HSP27, HSP60, HSP70, and HSP90: expression in bladder carcinoma. Cancer. 2003;98(5):970-977.
133. Elpek GO, Karaveli S, Simsek T, Keles N, Aksoy NH. Expression of heat-shock proteins hsp27, hsp70 and hsp90 in malignant epithelial tumour of the ovaries. APMIS. 2003;111(4):523-30.
134. Chiu CC, Lin CY, Lee LY, Chen YJ, Lu YC, Wang HM, et al. Molecular chaperones as a common set of proteins that regulate the invasion phenotype of head and neck cancer. Clin Cancer Res. 2011;17(14): 4629-41.
135. Albany C, Hahn NM. Heat shock and other apoptosis-related proteins as therapeutic targets in prostate cancer. Asian J Androl. 2014;16(3):359-63.

136. Cornford PA, Dodson AR, Parsons KF, Desmond AD, Woolfenden A, Fordham M, et al. Heat shock protein expression independently predicts clinical outcome in prostate cancer. Cancer Res. 2000;60(24): 7099-105.
137. Miyake H, Muramaki M, Kurahashi T, Takenaka A, Fujisawa M. Expression of potential molecular markers in prostate cancer: correlation with clinicopathological outcomes in patients undergoing radical prostatectomy. Urol Oncol. 2010;28(2): 145-51.
138. Taiyab A, Rao C. HSP90 modulates actin dynamics: inhibition of HSP90 leads to decreased cell motility and impairs invasion. Biochim Biophys Acta. 2011; 1813(1):213-21.
139. Kakeda M, Arock M, Schlapbach C, Yawalkar N. Increased expression of heat shock protein 90 in keratinocytes and mast cells in patients with psoriasis. J Am Acad Dermatol. 2014;70(4):683-90.
140. Pagetta A, Folda A, Brunati AM, Finotti P. Identification and purification from the plasma of Type 1 diabetic subjects of a proteolytically active Grp94Evidence that Grp94 is entirely responsible for plasma proteolytic activity. Diabetologia. 2003; 46(7):996-1006.
141. Hromadnikova I, Dvorakova L, Kotlabova K, Kestlerova A, Hympanova L, Novotna V et al. Assessment of placental and maternal stress responses in patients with pregnancy related complications via monitoring of heat shock protein mRNA levels. Mol Biol Rep. 2015;42(3):625-37.
142. Padmini E, Venkatraman U, Srinivasan L. Mechanism of JNK signal regulation by placental HSP70 and HSP90 in endothelial cell during preeclampsia. Toxicol Mech Methods. 2012;22(5):367-74.
143. Ekambaram P, Jayachandran T, Dhakshinamoorthy L. Differential expression of HSP90alpha and heme oxygenase in cord blood RBC during preeclampsia. Toxicol Mech Methods. 2013;23(2):113-19.
144. Zhang HH, Wang YP, Chen DB. Analysis of nitroso-proteomes in normotensive and severe preeclamptic human placentas. Biol Reprod. 2011;84(5):966-75.
145. Geller R, Taguwa S, Frydman J. Broad action of Hsp90 as a host chaperone required for viral replication. Biochim Biophys Acta. 2012;1823(3):698-706.
146. Carman A, Kishinevsky S, Koren J, III, Luo W, Chiosis G. Regulatory chaperone complexes in neurodegenerative diseases: A perspective on therapeutic intervention. Curr Alzheimer Res. 2014;11(1):59-68.
147. Uryu K, Richter-Landsberg C, Welch W, Sun E, Goldbaum O, Norris EH, et al. Convergence of heat shock protein 90 with ubiquitin in filamentous alpha-synuclein inclusions of alpha-synucleinopathies. Am J Pathol. 2006;168(3):947-61.
148. Dickey CA, Kamal A, Lundgren K, Klosak N, Bailey RM, Dunmore J, et al. The high-affinity HSP90-CHIP complex recognizes and selectively degrades phosphorylated tau client proteins. J Clin Invest. 2007;117(3):648-58.
149. Thompson AD, Scaglione KM, Prensner J, Gillies AT, Chinnaiyan A, Paulson HL et al. Analysis of the tau-associated proteome reveals that exchange of Hsp70 for Hsp90 is involved in tau degradation. ACS Chem Biol. 2012;7(10):1677-86.
150. Basso M, Samengo G, Nardo G, Massignan T, D'Alessandro G, Tartari S et al. Characterization of detergent-insoluble proteins in ALS indicates a causal link between nitrative stress and aggregation in pathogenesis. PLoS ONE. 2009;4(12): e8130.
151. Duval A, Olaru D, Campos L, Flandrin P, Nadal N, Guyotat D. Expression and prognostic significance of heat-shock proteins in myelodysplastic syndromes. Haematologica. 2006;91(5):713-14.
152. Flandrin-Gresta P, Solly F, Aanei CM, Cornillon J, Tavernier E, Nadal N, et al. Heat Shock Protein 90 is overexpressed in high-risk myelodysplastic syndromes and associated with higher expression and activation of Focal Adhesion Kinase. Oncotarget. 2012;3(10):1158-68.
153. Kasperkiewicz M, Tukaj S, Gembicki AJ, Sillo P, Gorog A, Zillikens D, et al. Evidence for a role of autoantibodies to heat shock protein 60, 70, and 90 in patients with dermatitis herpetiformis. Cell Stress Chaperones. 2014;19(6):837-43.
154. Pires ES, Khole VV. A block in the road to fertility: Autoantibodies to heat-shock protein 90-beta in human ovarian autoimmunity. Fertil Steril. 2009;92(4): 1395-409.
155. Yonekura K, Yokota S, Tanaka S, Kubota H, Fujii N, Matsumoto H, et al. Prevalence of anti-heat shock protein antibodies in cerebrospinal fluids of patients with

Guillain-Barre syndrome. J Neuroimmunol. 2004;156(1-2):204-9.
156. Harlow L, Rosas IO, Gochuico BR, Mikuls TR, Dellaripa PF, Oddis CV et al. Identification of citrullinated hsp90 isoforms as novel autoantigens in rheumatoid arthritis-associated interstitial lung disease. Arthritis Rheum. 2013;65(4):869-79.
157. Shelburne CE, Shelburne PS, Dhople VM, Sweier DG, Giannobile WV, Kinney JS, et al. Serum antibodies to Porphyromonas gingivalis chaperone HtpG predict health in periodontitis susceptible patients. PLoS ONE. 2008;3(4):e1984.
158. Workman P. Altered states: Selectively drugging the Hsp90 cancer chaperone. Trends Mol Med. 2004;10(2):47-51.
159. Nagy PD, Wang RY, Pogany J, Hafren A, Makinen K. Emerging picture of host chaperone and cyclophilin roles in RNA virus replication. Virology. 2011;411(2): 374-82.
160. Vashist S, Urena L, Gonzalez-Hernandez MB, Choi J, de RA, Rocha-Pereira J, et al. Molecular chaperone Hsp90 is a therapeutic target for noroviruses. J Virol. 2015;89(12):6352-63.
161. Das I, Basantray I, Mamidi P, Nayak TK, BMP, Chattopadhyay S, et al. Heat shock protein 90 positively regulates Chikungunya virus replication by stabilizing viral non-structural protein nsP2 during infection. PLoS ONE. 2014;9(6):e100531.
162. Gopalakrishnan R, Matta H, Chaudhary PM. A purine scaffold HSP90 inhibitor BIIB021 has selective activity against KSHV-associated primary effusion lymphoma and blocks vFLIP K13-induced NF-kappaB. Clin Cancer Res. 2013;19(18): 5016-26.
163. Geller R, Vignuzzi M, Andino R, Frydman J. Evolutionary constraints on chaperone-mediated folding provide an antiviral approach refractory to development of drug resistance. Genes Dev. 2007;21(2): 195-205.
164. Nakagawa S, Umehara T, Matsuda C, Kuge S, Sudoh M, Kohara M. Hsp90 inhibitors suppress HCV replication in replicon cells and humanized liver mice. Biochem Biophys Res Commun. 2007; 353(4):882-88.
165. Rathore AP, Haystead T, Das PK, Merits A, Ng ML, Vasudevan SG. Chikungunya virus nsP3 & nsP4 interacts with HSP-90 to promote virus replication: HSP-90 inhibitors reduce CHIKV infection and inflammation in vivo. Antiviral Res. 2014;103:7-16.
166. Nayar U, Lu P, Goldstein RL, Vider J, Ballon G, Rodina A, et al. Targeting the Hsp90-associated viral oncoproteome in gammaherpesvirus-associated malignancy. Blood. 2013;122(16):2837-47.
167. Nimmanapalli R, O'Bryan E, Bhalla K. Geldanamycin and its analogue 17-allylamino-17-demethoxygeldanamycin lowers Bcr-Abl levels and induces apoptosis and differentiation of Bcr-Abl-positive human leukemic blasts. Cancer Res. 2001;61(5): 1799-804.
168. Burch AD, Weller SK. Herpes simplex virus type 1 DNA polymerase requires the mammalian chaperone hsp90 for proper localization to the nucleus. J Virol. 2005; 79(16):10740-10749.
169. Basha W, Kitagawa R, Uhara M, Imazu H, Uechi K, Tanaka J. Geldanamycin, a potent and specific inhibitor of Hsp90, inhibits gene expression and replication of human cytomegalovirus. Antivir Chem Chemother. 2005;16(2):135-46.
170. Li YH, Tao PZ, Liu YZ, Jiang JD. Geldanamycin, a ligand of heat shock protein 90, inhibits the replication of herpes simplex virus type 1 in vitro. Antimicrob Agents Chemother. 2004;48(3):867-72.
171. Kyratsous CA, Silverstein SJ. BAG3, a host cochaperone, facilitates varicella-zoster virus replication. J Virol. 2007; 81(14):7491-503.
172. Castorena KM, Weeks SA, Stapleford KA, Cadwallader AM, Miller DJ. A functional heat shock protein 90 chaperone is essential for efficient flock house virus RNA polymerase synthesis in Drosophila cells. J Virol. 2007;81(16):8412-20.
173. Ujino S, Yamaguchi S, Shimotohno K, Takaku H. Heat-shock protein 90 is essential for stabilization of the hepatitis C virus nonstructural protein NS3. J Biol Chem. 2009;284(11):6841-46.
174. Neckers L. Hsp90 inhibitors as novel cancer chemotherapeutic agents. Trends Mol Med. 2002;8(4 Suppl):S55-S61.
175. Georgakis GV, Li Y, Younes A. The heat shock protein 90 inhibitor 17-AAG induces cell cycle arrest and apoptosis in mantle cell lymphoma cell lines by depleting cyclin D1, Akt, Bid and activating caspase 9. Br J Haematol. 2006;135(1):68-71.
176. Pacey S, Gore M, Chao D, Banerji U, Larkin J, Sarker S, et al. A Phase II trial of 17-allylamino, 17-demethoxygeldanamycin

(17-AAG, tanespimycin) in patients with metastatic melanoma. Invest New Drugs. 2012;30(1):341-49.

177. Gartner EM, Silverman P, Simon M, Flaherty L, Abrams J, Ivy P, et al. A phase II study of 17-allylamino-17-demethoxy-geldanamycin in metastatic or locally advanced, unresectable breast cancer. Breast Cancer Res Treat. 2012;131(3): 933-37.
178. Kummar S, Gutierrez ME, Gardner ER, Chen X, Figg WD, Zajac-Kaye M, et al. Phase I trial of 17-dimethylaminoethyl-amino-17-demethoxygeldanamycin (17-DMAG), a heat shock protein inhibitor, administered twice weekly in patients with advanced malignancies. Eur J Cancer. 2010;46(2):340-347.
179. Lancet JE, Gojo I, Burton M, Quinn M, Tighe SM, Kersey K, et al. Phase I study of the heat shock protein 90 inhibitor alvespimycin (KOS-1022, 17-DMAG) administered intravenously twice weekly to patients with acute myeloid leukemia. Leukemia. 2010;24(4):699-705.
180. Palacios C, Lopez-Perez AI, Lopez-Rivas A. Down-regulation of RIP expression by 17-dimethylaminoethylamino-17-demethoxygeldanamycin promotes TRAIL-induced apoptosis in breast tumor cells. Cancer Lett. 2010;287(2):207-15.
181. Ayrault O, Godeny MD, Dillon C, Zindy F, Fitzgerald P, Roussel MF, et al. Inhibition of Hsp90 via 17-DMAG induces apoptosis in a p53-dependent manner to prevent medulloblastoma. Proc Natl Acad Sci U S A. 2009;106(40):17037-42.
182. Chatterjee M, Jain S, Stuhmer T, Andrulis M, Ungethum U, Kuban RJ, et al. STAT3 and MAPK signaling maintain over-expression of heat shock proteins 90alpha and beta in multiple myeloma cells, which critically contribute to tumor-cell survival. Blood. 2007;109(2):720-728.
183. Dutta D, Chattopadhyay S, Bagchi P, Halder UC, Nandi S, Mukherjee A, et al. Active participation of cellular chaperone Hsp90 in regulating the function of rotavirus nonstructural protein 3 (NSP3). J Biol Chem. 2011;286(22):20065-77.
184. Chase G, Deng T, Fodor E, Leung BW, Mayer D, Schwemmle M, et al. Hsp90 inhibitors reduce influenza virus replication in cell culture. Virology. 2008;377(2):431-39.
185. Di K, Keir ST, exandru-Abrams D, Gong X, Nguyen H, Friedman HS, et al. Profiling Hsp90 differential expression and the molecular effects of the Hsp90 inhibitor IPI-504 in high-grade glioma models. J Neurooncol. 2014;120(3):473-81.
186. Modi S, Saura C, Henderson C, Lin NU, Mahtani R, Goddard J, et al. A multicenter trial evaluating retaspimycin HCL (IPI-504) plus trastuzumab in patients with advanced or metastatic HER2-positive breast cancer. Breast Cancer Res Treat. 2013;139(1): 107-13.
187. Sequist LV, von Pawel J, Garmey EG, Akerley WL, Brugger W, Ferrari D, et al. Randomized phase II study of erlotinib plus tivantinib versus erlotinib plus placebo in previously treated non-small-cell lung cancer. J Clin Oncol. 2011;29(24):3307-15.

Demetri GD, Le Cesne A, von Mehren M, Chmielowski B, Bauer S, Chow WA, et al. Final results from a phase III study of IPI-504 (retaspimycin hydrochloride) versus placebo in patients (pts) with gastro-intestinal stromal tumors (GIST) following failure of kinase inhibitor therapies. 2010 Gastrointestinal Cancers Symposium; 2010. Accessed 21st January 2016.
Available: http://meetinglibrary.asco.org/content/2285-72
188. Marcu MG, Chadli A, Bouhouche I, Catelli M, Neckers LM. The heat shock protein 90 antagonist novobiocin interacts with a previously unrecognized ATP-binding domain in the carboxyl terminus of the chaperone. J Biol Chem. 2000;275(47): 37181-86.
189. Samuels DS, Garon CF. Coumermycin A1 inhibits growth and induces relaxation of supercoiled plasmids in Borrelia burgdorferi, the Lyme disease agent. Antimicrob Agents Chemother. 1993;37(1): 46-50.
190. Mutsvunguma LZ, Moetlhoa B, Edkins AL, Luke GA, Blatch GL, Knox C. Theiler's murine encephalomyelitis virus infection induces a redistribution of heat shock proteins 70 and 90 in BHK-21 cells, and is inhibited by novobiocin and geldanamycin. Cell Stress Chaperones. 2011;16(5):505-15.
191. Sekiguchi J, Shuman S. Novobiocin inhibits vaccinia virus replication by blocking virus assembly. Virology. 1997;235(1):129-37.
192. Gonzalez-Molleda L, Wang Y, Yuan Y. Potent antiviral activity of topoisomerase I and II inhibitors against Kaposi's sarcoma-

associated herpesvirus. Antimicrob Agents Chemother. 2012;56(2):893-902.

193. Allan RK, Mok D, Ward BK, Ratajczak T. Modulation of chaperone function and cochaperone interaction by novobiocin in the C-terminal domain of Hsp90: Evidence that coumarin antibiotics disrupt Hsp90 dimerization. J Biol Chem. 2006;281(11): 7161-71.
194. Wu LX, Xu JH, Zhang KZ, Lin Q, Huang XW, Wen CX, et al. Disruption of the Bcr-Abl/Hsp90 protein complex: A possible mechanism to inhibit Bcr-Abl-positive human leukemic blasts by novobiocin. Leukemia. 2008;22(7):1402-9.
195. Shelton SN, Shawgo ME, Matthews SB, Lu Y, Donnelly AC, Szabla K, et al. KU135, a novel novobiocin-derived C-terminal inhibitor of the 90-kDa heat shock protein, exerts potent antiproliferative effects in human leukemic cells. Mol Pharmacol. 2009;76(6):1314-22.
196. Schulte TW, Akinaga S, Soga S, Sullivan W, Stensgard B, Toft D, et al. Antibiotic radicicol binds to the N-terminal domain of Hsp90 and shares important biologic activities with geldanamycin. Cell Stress Chaperones. 1998;3(2):100-108.
197. Higashi C, Saji C, Yamada K, Kagawa H, Ohga R, Taira T, et al. The effects of heat shock protein 90 inhibitors on apoptosis and viral replication in primary effusion lymphoma cells. Biol Pharm Bull. 2012; 35(5):725-30.
198. Smith DR, McCarthy S, Chrovian A, Olinger G, Stossel A, Geisbert TW et al. Inhibition of heat-shock protein 90 reduces *Ebola virus* replication. Antiviral Res. 2010;87(2):187-94.
199. Connor JH, McKenzie MO, Parks GD, Lyles DS. Antiviral activity and RNA polymerase degradation following Hsp90 inhibition in a range of negative strand viruses. Virology. 2007;362(1):109-19.
200. Shi X, Chen X, Li X, Lan X, Zhao C, Liu S et al. Gambogic acid induces apoptosis in imatinib-resistant chronic myeloid leukemia cells via inducing proteasome inhibition and caspase-dependent Bcr-Abl down-regulation. Clin Cancer Res. 2014;20(1): 151-63.
201. Zhang L, Yi Y, Chen J, Sun Y, Guo Q, Zheng Z, et al. Gambogic acid inhibits Hsp90 and deregulates TNF-alpha/NF-kappaB in HeLa cells. Biochem Biophys Res Commun. 2010;403(3-4):282-87.
202. Lv Y, Gong L, Wang Z, Han F, Liu H, Lu X, et al. Curcumin inhibits human cyto-megalovirus by downregulating heat shock protein 90. Mol Med Rep. 2015;12(3): 4789-93.
203. Angelo LS, Maxwell DS, Wu JY, Sun D, Hawke DH, McCutcheon IE, et al. Binding partners for curcumin in human schwannoma cells: Biologic implications. Bioorg Med Chem. 2013;21(4):932-39.
204. Giommarelli C, Zuco V, Favini E, Pisano C, Dal PF, De TN, et al. The enhancement of antiproliferative and proapoptotic activity of HDAC inhibitors by curcumin is mediated by Hsp90 inhibition. Cell Mol Life Sci. 2010;67(6):995-1004.
205. Wu LX, Wu Y, Chen RJ, Liu Y, Huang LS, Lou LG, et al. Curcumin derivative C817 inhibits proliferation of imatinib-resistant chronic myeloid leukemia cells with wild-type or mutant Bcr-Abl in vitro. Acta Pharmacol Sin. 2014;35(3):401-9.
206. James MI, Iwuji C, Irving G, Karmokar A, Higgins JA, Griffin-Teal N, et al. Curcumin inhibits cancer stem cell phenotypes in ex vivo models of colorectal liver metastases, and is clinically safe and tolerable in combination with FOLFOX chemotherapy. Cancer Lett. 2015;364(2):135-41.
207. Rechtman MM, Har-Noy O, Bar-Yishay I, Fishman S, Adamovich Y, Shaul Y, et al. Curcumin inhibits hepatitis B virus via down-regulation of the metabolic coactivator PGC-1alpha. FEBS Lett. 2010; 584(11):2485-90.
208. Kim K, Kim KH, Kim HY, Cho HK, Sakamoto N, Cheong J. Curcumin inhibits hepatitis C virus replication via suppressing the Akt-SREBP-1 pathway. FEBS Lett. 2010;584(4):707-12.
209. Chen MH, Lee MY, Chuang JJ, Li YZ, Ning ST, Chen JC, et al. Curcumin inhibits HCV replication by induction of heme oxygenase-1 and suppression of AKT. Int J Mol Med. 2012;30(5):1021-28.
210. Prasad S, Tyagi AK. Curcumin and its analogues: A potential natural compound against HIV infection and AIDS. Food Funct. 2015;6(11):3412-19.
211. Yin Z, Henry EC, Gasiewicz TA. (-)-Epigallocatechin-3-gallate is a novel Hsp90 inhibitor. Biochemistry. 2009;48(2):336-45.
212. Bhat R, Adam AT, Lee JJ, Gasiewicz TA, Henry EC, Rotella DP. Towards the discovery of drug-like epigallocatechin gallate analogs as Hsp90 inhibitors. Bioorg Med Chem Lett. 2014;24(10):2263-66.

213. Donnelly A, Blagg BS. Novobiocin and additional inhibitors of the Hsp90 C-terminal nucleotide-binding pocket. Curr Med Chem. 2008;15(26):2702-17.
214. Sawai A, Chandarlapaty S, Greulich H, Gonen M, Ye Q, Arteaga CL, et al. Inhibition of Hsp90 down-regulates mutant epidermal growth factor receptor (EGFR) expression and sensitizes EGFR mutant tumors to paclitaxel. Cancer Res. 2008; 68(2):589-96.
215. Solit DB, Basso AD, Olshen AB, Scher HI, Rosen N. Inhibition of heat shock protein 90 function down-regulates Akt kinase and sensitizes tumors to Taxol. Cancer Res. 2003;63(9):2139-44.
216. Bucur O, Stancu AL, Goganau I, Petrescu SM, Pennarun B, Bertomeu T, et al. Combination of bortezomib and mitotic inhibitors down-modulate Bcr-Abl and efficiently eliminates tyrosine-kinase inhibitor sensitive and resistant Bcr-Abl-positive leukemic cells. PLoS ONE. 2013;8(10):e77390.
217. Lu M, Wang X, Shen L, Jia J, Gong J, Li J, et al. Nimotuzumab plus paclitaxel and cisplatin as the 1st line treatment for advanced esophageal squamous cell cancer (ESCC): A single centre prospective phase II trial. Cancer Sci; 2016.
218. Huang KJ, Chen YC, El-Shazly M, Du YC, Su JH, Tsao CW, et al. 5-Episinuleptolide acetate, a norcembranoidal diterpene from the formosan soft coral Sinularia sp., induces leukemia cell apoptosis through Hsp90 inhibition. Molecules. 2013;18(3): 2924-33.
219. Sharp SY, Boxall K, Rowlands M, Prodromou C, Roe SM, Maloney A, et al. In vitro biological characterization of a novel, synthetic diaryl pyrazole resorcinol class of heat shock protein 90 inhibitors. Cancer Res. 2007;67(5):2206-16.
220. Shiotsu Y, Neckers LM, Wortman I, An WG, Schulte TW, Soga S, et al. Novel oxime derivatives of radicicol induce erythroid differentiation associated with preferential G(1) phase accumulation against chronic myelogenous leukemia cells through destabilization of Bcr-Abl with Hsp90 complex. Blood. 2000;96(6):2284-91.
221. Bussenius J, Blazey CM, Aay N, Anand NK, Arcalas A, Baik T, et al. Discovery of XL888: a novel tropane-derived small molecule inhibitor of HSP90. Bioorg Med Chem Lett. 2012;22(17):5396-404.
222. Hong DS, Banerji U, Tavana B, George GC, Aaron J, Kurzrock R. Targeting the molecular chaperone heat shock protein 90 (HSP90): lessons learned and future directions. Cancer Treat Rev. 2013;39(4): 375-87.
223. Haarberg HE, Paraiso KH, Wood E, Rebecca VW, Sondak VK, Koomen JM, et al. Inhibition of Wee1, AKT, and CDK4 underlies the efficacy of the HSP90 inhibitor XL888 in an in vivo model of NRAS-mutant melanoma. Mol Cancer Ther. 2013;12(6):901-12.
224. Nagaraju GP, Park W, Wen J, Mahaseth H, Landry J, Farris AB, et al. Antiangiogenic effects of ganetespib in colorectal cancer mediated through inhibition of HIF-1alpha and STAT-3. Angiogenesis. 2013;16(4):903-17.
225. Shimamura T, Perera SA, Foley KP, Sang J, Rodig SJ, Inoue T, et al. Ganetespib (STA-9090), a nongeldanamycin HSP90 inhibitor, has potent antitumor activity in in vitro and in vivo models of non-small cell lung cancer. Clin Cancer Res. 2012;18(18):4973-85.
226. Brough PA, Aherne W, Barril X, Borgognoni J, Boxall K, Cansfield JE, et al. 4,5-diarylisoxazole Hsp90 chaperone inhibitors: potential therapeutic agents for the treatment of cancer. J Med Chem. 2008;51(2):196-218.
227. Eccles SA, Massey A, Raynaud FI, Sharp SY, Box G, Valenti M, et al. NVP-AUY922: a novel heat shock protein 90 inhibitor active against xenograft tumor growth, angiogenesis, and metastasis. Cancer Res. 2008;68(8):2850-2860.
228. Taniguchi H, Hasegawa H, Sasaki D, Ando K, Sawayama Y, Imanishi D, et al. Heat shock protein 90 inhibitor NVP-AUY922 exerts potent activity against adult T-cell leukemia-lymphoma cells. Cancer Sci. 2014;105(12):1601-8.
229. Tao W, Chakraborty SN, Leng X, Ma H, Arlinghaus RB. HSP90 inhibitor AUY922 induces cell death by disruption of the Bcr-Abl, Jak2 and HSP90 signaling network complex in leukemia cells. Genes Cancer. 2015;6(1-2):19-29.
230. Garon EB, et al., Phase II study of the HSP90 inhibitor AUY922 in patients with previously treated, advanced non-small cell lung cancer (NSCLC). J Clin Oncol. 2012;30(15_suppl):7543.

231. Kong A, et al. Phase IB/II study of the HSP90 inhibitor AUY922, in combination with trastuzumab, in patients with HER2+ advanced breast cancer. J Clin Oncol. 2012;30(15_suppl):530.
232. Johnson ML, et al. A phase II study of HSP90 inhibitor AUY922 and erlotinib (E) for patients (pts) with EGFR-mutant lung cancer and acquired resistance (AR) to EGFR tyrosine kinase inhibitors (EGFR TKIs). 2013;8036.
233. Sharp SY, Prodromou C, Boxall K, Powers MV, Holmes JL, Box G, et al. Inhibition of the heat shock protein 90 molecular chaperone in vitro and in vivo by novel, synthetic, potent resorcinylic pyrazole/ isoxazole amide analogues. Mol Cancer Ther. 2007;6(4):1198-211.
234. Lundgren K, Zhang H, Brekken J, Huser N, Powell RE, Timple N, et al. BIIB021, an orally available, fully synthetic small-molecule inhibitor of the heat shock protein Hsp90. Mol Cancer Ther. 2009;8(4):921-29.
235. Boll B, Eltaib F, Reiners KS, von TB, Tawadros S, Simhadri VR, et al. Heat shock protein 90 inhibitor BIIB021 (CNF2024) depletes NF-kappaB and sensitizes Hodgkin's lymphoma cells for natural killer cell-mediated cytotoxicity. Clin Cancer Res. 2009;15(16):5108-16.
236. Zhang H, Neely L, Lundgren K, Yang YC, Lough R, Timple N, et al. BIIB021, a synthetic Hsp90 inhibitor, has broad application against tumors with acquired multidrug resistance. Int J Cancer. 2010; 126(5):1226-34.
237. Saif MW, Takimoto C, Mita M, Banerji U, Lamanna N, Castro J, et al. A phase 1, dose-escalation, pharmacokinetic and pharmacodynamic study of BIIB021 administered orally in patients with advanced solid tumors. Clin Cancer Res. 2014;20(2):445-55.
238. Dickson MA, Okuno SH, Keohan ML, Maki RG, D'Adamo DR, Akhurst TJ, et al. Phase II study of the HSP90-inhibitor BIIB021 in gastrointestinal stromal tumors. Ann Oncol. 2013;24(1):252-57.
239. Huang KH, Veal JM, Fadden RP, Rice JW, Eaves J, Strachan JP, et al. Discovery of novel 2-aminobenzamide inhibitors of heat shock protein 90 as potent, selective and orally active antitumor agents. J Med Chem. 2009;52(14):4288-305.
240. Murshid A, Gong J, Stevenson MA, Calderwood SK. Heat shock proteins and cancer vaccines: developments in the past decade and chaperoning in the decade to come. Expert Rev Vaccines. 2011;10(11): 1553-68.
241. Wang S, Wang X, Du Z, Liu Y, Huang D, Zheng K, et al. SNX-25a, a novel Hsp90 inhibitor, inhibited human cancer growth more potently than 17-AAG. Biochem Biophys Res Commun. 2014.
242. Rajan A, Kelly RJ, Trepel JB, Kim YS, Alarcon SV, Kummar S, et al. A phase I study of PF-04929113 (SNX-5422), an orally bioavailable heat shock protein 90 inhibitor, in patients with refractory solid tumor malignancies and lymphomas. Clin Cancer Res. 2011;17(21):6831-39.
243. Reddy N, Voorhees PM, Houk BE, Brega N, Hinson JM Jr, Jillela A. Phase I trial of the HSP90 inhibitor PF-04929113 (SNX5422) in adult patients with recurrent, refractory hematologic malignancies. Clin Lymphoma Myeloma Leuk. 2013;13(4): 385-91.
244. Plescia J, Salz W, Xia F, Pennati M, Zaffaroni N, Daidone MG, et al. Rational design of shepherdin, a novel anticancer agent. Cancer Cell. 2005;7(5):457-68.
245. Stella S, Tirro E, Conte E, Stagno F, Di RF, Manzella L, et al. Suppression of survivin induced by a BCR-ABL/JAK2/ STAT3 pathway sensitizes imatinib-resistant CML cells to different cytotoxic drugs. Mol Cancer Ther. 2013;12(6):1085-98.
246. Maroney AC, Marugan JJ, Mezzasalma TM, Barnakov AN, Garrabrant TA, Weaner LE, et al. Dihydroquinone ansamycins: toward resolving the conflict between low in vitro affinity and high cellular potency of geldanamycin derivatives. Biochemistry. 2006;45(17):5678-85.
247. Sydor JR, Normant E, Pien CS, Porter JR, Ge J, Grenier L, et al. Development of 17-allylamino-17-demethoxygeldanamycin hydroquinone hydrochloride (IPI-504), an anti-cancer agent directed against Hsp90. Proc Natl Acad Sci U S A. 2006;103(46): 17408-13.
248. Jhaveri K, Chandarlapaty S, Lake D, Gilewski T, Robson M, Goldfarb S, et al. A phase II open-label study of ganetespib, a novel heat shock protein 90 inhibitor for patients with metastatic breast cancer. Clin Breast Cancer. 2014;14(3):154-60.
249. Fadden P, Huang KH, Veal JM, Steed PM, Barabasz AF, Foley B, et al. Application of

chemoproteomics to drug discovery: identification of a clinical candidate targeting hsp90. Chem Biol. 2010;17(7): 686-94.
250. Modi S, Stopeck A, Linden H, Solit D, Chandarlapaty S, Rosen N, et al. HSP90 inhibition is effective in breast cancer: A phase II trial of tanespimycin (17-AAG) plus trastuzumab in patients with HER2-positive metastatic breast cancer progressing on trastuzumab. Clin Cancer Res. 2011;17(15):5132-39.
251. Schilling D, Kuhnel A, Konrad S, Tetzlaff F, Bayer C, Yaglom J, et al. Sensitizing tumor cells to radiation by targeting the heat shock response. Cancer Lett. 2015;360(2): 294-301.
252. Aggarwal BB, Gehlot P. Inflammation and cancer: how friendly is the relationship for cancer patients? Curr Opin Pharmacol. 2009;9(4):351-69.
253. Chendil D, Ranga RS, Meigooni D, Sathishkumar S, Ahmed MM. Curcumin confers radiosensitizing effect in prostate cancer cell line PC-3. Oncogene. 2004;23(8):1599-607.
254. Hoffmann J, Junker H, Schmieder A, Venz S, Brandt R, Multhoff G, et al. EGCG downregulates IL-1RI expression and suppresses IL-1-induced tumorigenic factors in human pancreatic adeno-carcinoma cells. Biochem Pharmacol. 2011;821153-62.
255. Zhang G, Wang Y, Zhang Y, Wan X, Li J, Liu K, et al. Anti-cancer activities of tea epigallocatechin-3-gallate in breast cancer patients under radiotherapy. Curr Mol Med. 2012;12(2):163-76.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/13840