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# Heat Shock Proteins: Their Role in Tumor Development and Their Therapeutic Applications Against Cancer

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**Abstract**
Exposure of cells to conditions of environmental stress (e.g. Heat shock) results in the inducible expression of heat shock proteins that function as molecular chaperones or proteases. Heat shock proteins have been classified into six major families according to their molecular size: Hsp100, Hsp90, Hsp70, Hsp60, Hsp40, and small heat shock proteins. Heat shock proteins (HSPs) function by inducing an ATPase-coupled structural change, followed by interactions with diverse co-chaperones and over 200 client proteins implicated in many critical signaling networks. These highly expressed HSPs participate in the onset and progression of several human diseases including cancer, and their connection with tumorigenesis has facilitated research and clinical trials related to targeting HSPs as a novel anti-tumor therapy. The predominant mechanism of chaperone inhibition is through either disruption of the HSP association with client protein or an altered binding state that ultimately leads to proteasome-mediated degradation. Importantly, chaperone inhibition results in the degradation of several client proteins that play critical roles in many of the pathways known as the Hallmarks of Cancer, such as proliferation, angiogenesis, invasion, metastasis, and drug resistance. Hypoxia and other stressful stimuli induce HIF expression as well as subsequent cellular response, resulting in a cascade of signaling events that induce VEGF expression and angiogenesis. Importantly, several critical mediators in this angiogenic signaling pathway, including HIF, VEGF-receptor and IL-8/NF-κB are dependent upon Hsp90 for their function. Given that HSP is required for activation of VEGFR, PKB, and NFκB, HSP inhibitors can be employed to target multiple signaling molecules of angiogenesis pathway, as demonstrated by the potent suppression of VEGF and NO release both *in vitro* and *in vivo* with the overall outcome of inhibiting tumor angiogenesis. HSPs also participate in invasion and metastasis of tumor so the inhibition of HSPs through inhibiting client proteins can also be used as complementary to tumor therapy.
**Keywords**: Angiogenesis, heat shock proteins, invasion, metastasis, tumor, tumor therapy

## INTRODUCTION

Exposure of cells to conditions of environmental stress— including heat shock, oxidative stress, heavy metals, or pathologic conditions, such as ischemia and reperfusion, inflammation, tissue damage, infection, and mutant proteins associated with genetic diseases—results in the inducible expression of heat shock proteins that function as molecular chaperones or proteases. Molecular chaperones function in protein folding, translocation, and refolding of intermediates, proteases, such as the ubiquitin-dependent proteasome, ensure that damaged and short-lived proteins are degraded efficiently. Consequently, heat shock proteins assist in the recovery from stress either by repairing damaged proteins (protein refolding) or by degrading them, thus restoring protein homeostasis and promoting cell survival (Jolly and Morimoto, 2000).

The primary function of the immune response is to distinguish between molecules, usually proteins, which are construed as either component of self or non-self molecules likely derived from invading organisms. Through the mechanisms of central and peripheral tolerance, the immune response is deterred from attacking cells recognized as self (Walker and Abbas, 2002). The case of tumor immunity is however more ambiguous. A depressing variety of mechanisms have been found which may account for the ability of tumors to dismiss the attentions of the immune response. These include a "loss-of-self " mechanism in which major histocompatibility class I antigens cease to be expressed on the tumor cell surface, thus masking the presence of the tumor proteome and evading CD8+ killing (Moller and Hammerling, 1992). Additional mechanisms include the expression by cancer cells of Fas ligand that can recognize the presence of proapoptotic Fas on the tumor cell surface and trigger programmed cell death of CTL (Chouaib *et al*., 2002). In addition, the nonmalignant cell populations that migrate into the tumor microenvironment appear to play a key role in deterring immunity (Marigo *et al*., 2008; Terabe *et al*., 2005; Pekarek *et al*., 1995). Although cytotoxic CD8+ cells progress to and arrest at the periphery of many tumors, crossing the tumor capillary wall comprises a barrier to entry of such cells; indeed, ability of CTL to penetrate tumors is a favorable prognostic feature (Liu *et al*., 2012; Fridman *et al*., 2011; Galon *et al*., 2006). As mentioned, however, tumors also attract a range of normal cells in a process that resembles a normal wound-healing response. Invading cells include regulatory T lymphocytes (Treg), primary players in peripheral tolerance and in the defense against autoimmunity (Beyer *et al*., 2012; Schmidt *et al*., 2012). Treg exhibit multiple immunosuppressive mechanisms including the secretion of cytokines such as transforming growth factor beta (TGF$\beta$) and interleukin-10 (IL-10), the killing of CTL, and inhibition of immune cells through a cell contact mechanism (Schmidt *et al*., 2012). It may be significant that cancer stem cells (CSCs) that account for tumor initiation, metastasis, and resistance to many forms of therapy can attract Treg and lead to the expression of

immunosuppressive IL-10 (Schatton and Frank, 2009; Visvader, 2009).

Another class of immunoregulatory cells associated with tumors includes myeloid-derived suppressor cells (MDSCs). MDSCs are a heterogeneous population of early myeloid precursors, immature granulocytes, macrophages, and DC that can suppress CD4+ and CD8+ T cells, NK and NKT cells and promote development of Treg by multiple mechanisms (Marigo *et al*., 2008). In addition, many tumors contain tumor associated macrophages (TAMs) that are also suppressors of tumor immunity through production of IL-10, stimulation of Treg, and synthesis of the co-inhibitory factor CTLA-4. Also attracted to the tumor are mesenchymal stem cells (MSCs) that can give rise to a tumor-associated fibroblast (TAF) population that supplies growth factors such as FGF, TGF*β*, and VEGF required for growth and angiogenesis (Kraman *et al*., 2010). In order to survive under the harsh conditions within the tumor microenvironment, cancer cells typically become dependent on stress-inducible HSPs to become refractory to chemotherapy, tolerant to hypoxia, resistant to apoptosis, and to suppress antitumor immunity, all the while acquiring the properties of invasiveness and metastasis during cancer progression. All the above mentioned phenomena in tumor *milieu* use HSPs directly or indirectly or HSPs are implicated in cancer environment development and have been shown to specifically interfere with current antitumor therapies. Not surprisingly, inhibition of HSP's ATPase activity and disruption of ongoing chaperone folding cycles results in dissociation, destabilization, and proteasomal degradation of a variety of client proteins, including the cancer-associated targets Bcl-2, Apaf-1, PKB, and MMP-2 (Jego *et al*., 2010; Wang *et al*., 2009). Therefore, the objectives of this seminar paper are to review on: the connection between molecular chaperones and tumorigenesis, especially focusing on the roles of the molecular chaperone families Hsp90 and Hsp70, and the therapeutic application of heat shock proteins against cancer.

## GENERAL FEATURES OF HEAT SHOCK PROTEINS

### Types of heat shock proteins

Heat shock proteins have been classified into six major families according to their molecular size: Hsp100, Hsp90, Hsp70, Hsp60, Hsp40, and small heat shock proteins (Jolly and Morimoto, 2000).

*Hsp70 Family*

Seventy kilo Dalton heat shock protein (Hsp70) family members are not only responsible for protein conformational assembly, but also preventing protein misfolding and aggregation during a variety of post-translational processes including protein targeting and degradation, membrane translocation, and apoptosis. The Hspα group of HSPs includes Hsp70, Hsp71, Hsp72, and GRP78 (BiP). The members of this Hsp70 family represent the most highly conserved molecular chaperones. They have two major functional domains: an N-terminal ATPase-binding domain (ABD) responsible for substrate binding and refolding, and a C-terminal peptide-binding domain (PBD) to facilitate the release of client protein after ATP hydrolysis. The 78-kDa glucose regulated protein (GRP78), also well known as immunoglobulin interacting heavy chain binding protein (BiP), was originally found as a major protein for maintaining intracellular homeostasis in endoplasmic reticulum (ER) called the unfolded protein response (UPR). High level of GRP78 confers multiple survival advantages to facilitate the proliferation of cancer cells through harsh conditions and to acquire chemotherapeutic resistance (Gonzalez-Gronow *et al*., 2009; Dong *et al*., 2008; Lee, 2007; Li and Lee, 2006; Lee, 2001).

While it is difficult to detect GRP78 expression in normal cells, over-expression of GRP78 can be detected in many tumor cell lines and primary tumors, such as breast and prostate cancer cells. *In vivo* studies demonstrate a critical role of GRP78 in tumor growth, metastasis, and angiogenesis in xenograft models and in the GRP78 heterozegous mice with partial reduction of GRP78 (Lee, 2007). GRP78 can be detected as a cell surface protein in a broad variety of tumor cells by global profiling of the cell surface proteins, suggesting cancer cells may have evolved a specific mechanism for presenting GRP78 epitopes on the cell surface (Misra and Pizzo, 2010; Gonzalez-Gronow *et al*., 2009; Misra *et al*., 2006).

Seventy two kilo Dalton heat shock protein (Hsp72) is another major heat shock-induced protein capable of protecting cells from stressful conditions. Hsp72 can be present at elevated levels in various forms of tumors and in many transformed cell lines. Based on more novel findings of roles of Hsp70 from clinical and basic research, Hsp70 targeted therapy is clearly an attractive approach for anti-tumor treatment. Considerable progress has been made in targeting Hsp70 using small molecule inhibitors in cancer as well as in other protein folding diseases (Li, 2011).

*Hsp90 Family*

The most prevalent members of the Hsp90 family are Hsp90α and Hsp90β isoforms which are expressed by two distinct genes whose protein products are mainly cytoplasmic. So far only Hsp90α has been reported to stabilize MMP-2 and prevents it from degradation in cancer cells through the interaction between the Hsp90α middle domain and the MMP-2 C-terminal hemopexin domain (Song *et al*., 2010).

Ninety kilo Dalton heat shock protein inhibition has attracted considerable attention in the past two

 

decades as a promising approach for cancer therapy, leading to degradation of multiple oncogenic client proteins. A number of compounds and their derivatives have been shown to bind the ATP binding pocket of Hsp90, which prevents ATP hydrolysis and blocks protein folding and assembly. Instead, Hsp90 inhibition results in the targeting of its client proteins to a proteosomal degradation pathway (Tapia and Morano, 2010).

**Table 1**. Brief summary of the nomenclature, location, and function of the major heat shock protein families.

| Family | Organism | Location | Functions |
|---|---|---|---|
| Hsp100 | *E. coli* | Cytosol | Role in stress tolerance; helps the resolubilization of heat-inactivated proteins from insoluble aggregates |
| | *S. cerevisiae* | Cytosol | |
| Hsp90 | *E. coli* | Cytosol | Role in signal transduction (e.g., interaction with steroid hormone receptors, tyrosine kinases, serine/ threonine kinases); refolds and maintains proteins *in vitro*; autoregulation of the heat shock response; role in cell cycle and proliferation |
| | *S. cerevisiae* | Cytosol | |
| | Mammals | Cytosol/ER | |
| Hsp70 | *E. coli* | Cytosol | Roles in lambda phage replication; autoregulation of the heat shock response; interaction with nascent chain polypeptides; functions in interorganellar transport; roles in signal transduction; refolds and maintains denatured proteins *in vitro*; role in cell cycle and proliferation; antiapoptotic activity; potential antigen-presenting molecule in tumor cells. |
| | *S. cerevisiae* | Cytosol/ER/ Mitochondria | |
| | Mammals | Cytosol/nucleus/ER/ Mitochondria | |
| Hsp60 | *E. coli* | Cytosol | Refolds and prevents aggregation of denatured proteins *in vitro*; may facilitate protein degradation by acting as a cofactor in proteolytic systems; role in the assembly of bacteriophages and Rubisco (an abundant protein in the chloroplast) |
| | *S. cerevisiae* | Mitochondria | |
| | Plants | Chloroplasts | |
| | Mammals | Mitochondria | |
| Hsp40 | *E. coli* | Cytosol | Essential cochaperone activity with Hsp70 proteins to enhance rate of adenosine triphosphatease activity and substrate release |
| | *S. cerevisiae* | Cytosol/nucleus | |
| | Mammals | | |
| Small HSPs | *E. coli* | Cytosol | Suppresses aggregation and heat inactivation of proteins *in vitro*; confers thermotolerance through stabilization of microfilaments; antiapoptotic activity |
| | *S. cerevisiae* | Cytosol | |
| | Mammals | Cytosol | |

**Source**: (Jolly and Morimoto, 2000).

**Synthesis of heat shock proteins**

Heat shock proteins are vital throughout the whole lifetime of our cells. However, they are needed even more after environmental stress, which induces protein damage. In eukaryotic organisms the expression of heat shock protein messenger RNA-s is mediated by a family of transcription factors, called heat shock factors. The synthesis of these proteins is under control of at least four different heat shock factors (HSF1-4). Heat shock factor 1 (HSF-1) plays a major role in heat shock response, while other members of the family are activated after prolonged stress, or participate in processes such as embryonic development, or cell differentiation. After stress, damaged proteins become abundant and liberate the heat shock factor from its Hsp70/Hsp90 complexes. This process sets the stage for the trimerization, nuclear translocation and phosphorylation of HSF-1, which are all pre requisites for its binding to the special nucleotide segments, called heat shock elements, in the promoter region of heat shock protein genes. All these steps are modulated by numerous co-chaperones of the major heat shock proteins, Hsp70 and Hsp90, and most probably by other proteins as well (Morimoto, 1999).

Following stress body selectively increases some proteins like HSPs synthesis and decreases other proteins synthesis i.e. during stress, all the subsequent steps of protein synthesis (RNA splicing, nuclear export and translation itself) are blocked and heat shock RNA-s developed various strategies to circumvent these problems. Primary transcripts (such as Hsp70 RNA) usually do not contain introns, or the open reading frame encoding the protein itself begins after the intron and the initialization may proceed from the intron as well (e.g. Hsp90) (Csermely *et al*., 1998).

Genes encoding HSPs are transcriptionally regulated by variety of physiologic processes including the cell cycle, cell proliferation, and differentiation (Jerome *et al*., 1993). These observations have led to suggestions that Hsp70 and Hsp90 may also have critical functions during cell growth, specifically associated with the cell cycle and the proliferative response (Aligue *et al*., 1994).

## ROLE OF HEAT SHOCK PROTEINS IN CANCER DEVELOPMENT

In transformed cells, mechanisms for controlling protein aggregation are critical for preventing cell death that can be induced by the increase in cell stress and the ultimate loss of cellular homeostasis. Interestingly, these pathways are interrelated with an underlying theme: cancer initiation and progression (Li, 2011). Hsp70 and Hsp90 were known to act as anti-apoptotic factors. Hsp90 participates in many key processes in oncogenesis such as self-sufficiency in growth signals, stabilization of mutant proteins, angiogenesis and metastasis (Laudanski and Wyczechowska, 2006).

### Function of heat shock proteins in angiogenesis

High levels of HSP expression are important for cancer cells to survive in a hypoxic tumor microenvironment, which is generally attributed to their effects on the transcription factor HIF-1α by mediating its stabilization and/or aggregation. HIF-1 is a heterodimer that is composed of both HIF-1α (120 kD) and HIF-1β (91-94 kD). HIF-1α is stabilized by Hsp90 in hypoxic conditions and normally degraded by prolyl hydroxylase (PHD), the von Hipple-Lindau (VHL)/Elongin-C/Elongin-B E3 ubiquitin ligase complex through proteasomes dependent manner (Isaacs *et al.,* 2003).

Ninety kilo Dalton heat shock protein plays a critical role in structural modulation of oncoproteins including PKB and BCR-ABL. Hypoxia and other stressful stimuli induce HIF expression as well as subsequent cellular response, resulting in a cascade of signaling events that induce VEGF expression and angiogenesis. Importantly, several critical mediators in this angiogenic signaling pathway, including HIF, VEGF-receptor and IL-8/NF-κB are dependent upon Hsp90 for their function. Receptor Tyrosine Kinase (RTK) activation also potentially induces HIF expression via PKB/mTOR-mediated translation pathway. RTKs additionally transactivate Ephrin type-A receptor 2 (EphA2), a novel Hsp90 client protein known to be involved in tumor angiogenesis. In addition, HIF also promotes the expression of several RTK ligands, for example, hepatocyte growth factor (HGF) and TGF-α, as well as RTK receptors including endothelia growth factor receptor (EGFR) and insulin-like growth factor receptor (IGFR), thereby reinforcing these signaling interactions. Moreover, Hsp90 plays a role in NF-κB-induced VEGF expression and regulates downstream effectors. Given that Hsp90 is required for activation of VEGFR, PKB and NF-κB, Hsp90 inhibitors can be employed to target multiple signaling molecules of angiogenesis pathway, as demonstrated by the potent suppression of VEGF and NO release both *in vitro* and *in vivo* with the overall outcome of inhibiting tumor angiogenesis (Bohonowych *et al*., 2010).

In tumor cells, Signal Transducer and Activator of Transcription-3 (STAT3) is commonly activated and blocks apoptosis as wells promotes cell transformation. It is showed that Hsp90 inhibitors can also disrupt HIF-1α/STAT3 mediated autocrine loop for IL-6 and IGF-I in pancreatic adenocarcinoma cells and the highly metastatic pancreatic cells by direct disturbance with the functions of HIF-1α and STAT3. In the same study, ELISA data also demonstrated a marked reduction of VEGF-A expression after treatment with 17-allylamino-geldanamycin (17-AAG), a Hsp90 inhibitor (Li, 2011). Much of the known roles of Hsp90 in angiogenesis are shown schematically in Figure 1.

 

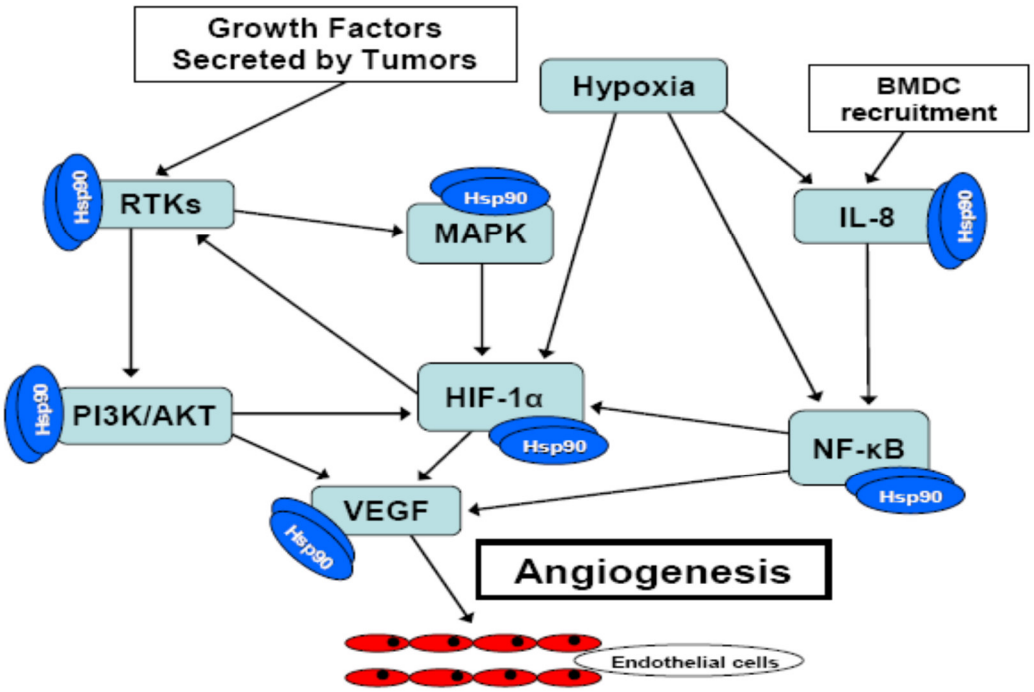


**Figure 1.** Hsp90 inhibitors targeting angiogenic signaling network in cancer**.**
**Source**: (Li, 2011)

**Function of heat shock proteins in invasion and metastasis**

Over-expression of heat shock factor 1 (HSF1) and HSPs in tumor cells displayed an increasing trend to invade tumor microenvironment and metastasize to distant sites, though molecular mechanisms have not yet fully understood. In addition to transcriptional regulation of HSPs expression, recent data also showed that HSF1 is an important facilitator for tumor progression. Accumulating research suggests that highly expressed downstream factors of HSF1, including Hsp27 and Hsp70, in tumor cells are at least partially responsible for the invasive and/or metastatic properties of tumors (Ciocca and Calderwood, 2005)

Ninety kilo Dalton heat shock protein was detected on the cell surface and in conditioned medium of tumor cells, where it acted as a molecular chaperone to assist in the activation of matrix metalloproteinase-2 (MMP-2), working with a complex of co-chaperone proteins including Hsp90 organizing protein (HOP), leading to elevated tumor invasiveness. Accumulating evidences indicate that Hsp90 especially Hsp90α can be expressed and function in the extracellular space acting as a molecular chaperone that assist in the maturation of pro-MMP-2 to its active form by stimulating propeptide cleavage. Activated MMP-2 protease digests many of the major extracellular matrix (ECM) components surrounding tumor tissue including fibronectin, laminins, collagens, *etc* thereby facilitating tumor invasion process (Li, 2011).

**HEAT SHOCK PROTEIN INHIBITION AS A CANCER TREATMENT**

Cancer is a threat both for human and animal life and is, thus, one of the least understood diseases having no effective therapy.Genetic and epigenetic changes characteristic of carcinogenesis make cancer cells antigenically distinct from normal human cells. Cancer cells express tumor-specific antigens and tumor-associated antigens (Mosolits *et al*., 2005). At the end of the 1980s, Hsp70, Hsp90, and GRP94 were identified as tumor-specific antigens expressed on the surface of various tumor cells and are, therefore, ideal targets for antitumor therapy (Konno *et al*., 1989)..

**Targeting Hsp90-mediated transformational signaling pathways**

Researchers have proposed the six hallmarks of cancer a decade ago to provide a logical and solid framework for understanding the biology of cancer. Two emerging hallmarks including reprogramming of energy metabolism and evading immune destruction have become additional highlights in the study of tumorigenesis. In addition, two enabling characteristics including tumor-promoting inflammation as well as genome instability and mutation have been proved to enhance the six core and emerging hallmark capabilities (Li, 2011).

More importantly, in clinic pathological term “desmoplasia” initially describing the growth of fibrous or connective tissue around the tumor lesion has been proven and investigated in the mechanistic study of tumor

 

progression, which further completes our overall consideration of cancer cells not as an "isolated island", which means they have to communicate with and depend on surrounding non-cancerous cells to facilitate tumor microenvironment. Microcommunity of cancer is not simply understood by passive composition of bystanders but a dynamic microenvironment communication of multiple cell types reciprocally interacting with each other to facilitate tumor progression, especially tumor-associated fibroblasts or cancer-associated fibroblasts (TAFs or CAFs), a major and critical component of tumor stroma tissue (Tlsty and Coussens 2006; Beacham and Cukierman, 2005; Kunz-Schughart and Knuechel, 2002;).

Appreciation of the critical hallmarks and characteristics for the development of cancer will definitely modulate the future direction of cancer research and promote our exploration of anti-tumor therapy with potential paradigm-shifting strategies. As shown in Figure 1, Hsp90 plays a multifaceted part involved in the acquisition and development of the hallmarks of cancer through interacting with many client proteins responsible for essential oncogenic transformation. If these client proteins fail to bind a specific ligand or receptor to form a meta-stable chaperone-client complex, then they are subjected to ubiquitination and finally degraded by proteasome, providing the major theoretical basis of pharmaceutical inhibition of Hsp90-mediated oncogenic signaling pathways. For example, Hsp90 inhibitor was reported to inhibit angiogenic signaling pathways either through HIF-dependent or -independent manner in the tumor vascularization process (Li, 2011).

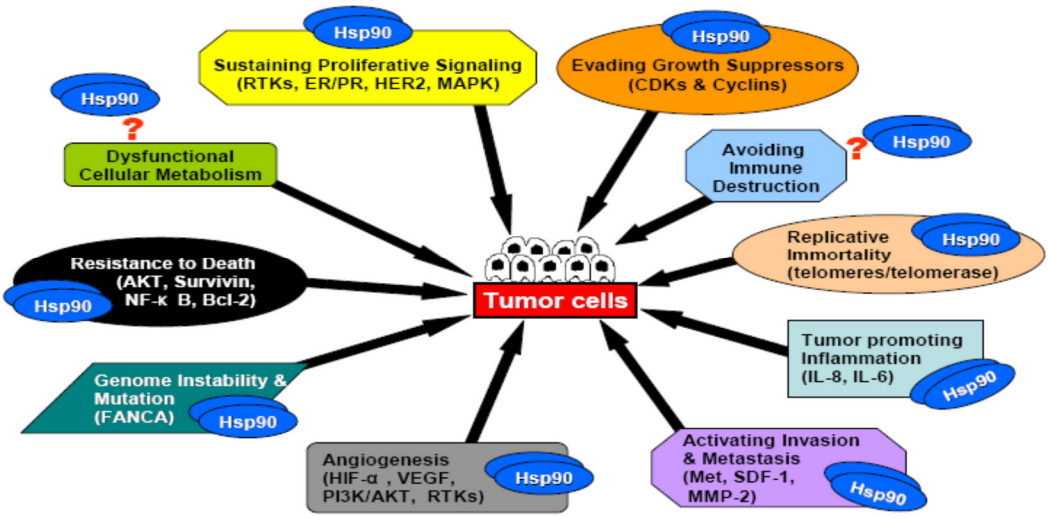


**Figure 2.** Current therapeutic targeting of the hallmarks of cancer using Hsp90 inhibitors.
**Source**: (Li, 2011)

Tumor cells activate endothelial cells through secretion of various proangiogenic growth factors including VEGF, COX-2, HGF, as well as FGF, which are regulated by hypoxia through the hypoxia inducible factor (HIF) and bind to corresponding RTK on dormant endothelial cells. Once endothelial cells become activated, they migrate and proliferate to form novel branches from the preexisting blood vessels by secreting the matrix metalloproteinases to detach from the extracellular matrix and basement membrane (Li, 2011).

Major anti-angiogenic effects of Hsp90 inhibitors are most likely associated with down-regulation of HIF activity, since the half life of HIF-1α is also controlled in an oxygen-independent way by the competitive binding of either Hsp90 to stabilize the protein, or the anchoring protein Receptor for activated C kinase 1 (RACK1) to interact with Elongin C and mediate prolyl hydroxylase domain protein 2 (PHD2) and von Hippel-Lindau protein (VHL)-independent ubiquitination and degradation of HIF-1α. IL-8/NF-κB signaling axis has been reported to upregulate VEGF expression through HIF-independent proangiogenic processes, while suppression of NF-κB signaling in animal model of ovarian cancer destroyed tumor angiogenesis with suppression of VEGF and IL-8 (Huang and Ingber, 2000).

Furthermore, Hsp90 inhibitors-mediated suppression of NF-κB was observed in several studies. Taken together, these data suggested that Hsp90 inhibition reduced NF-κB activation in tumor angiogenesis through IL-8 mediated signaling pathway. Given that the majority of these oncogenic proteins identified as Hsp90 client proteins, Hsp90-directed pharmacological intervention becomes promising for broad suppression of these signaling interactions within the tumor progression (Li, 2011).

Tumor-associated fibroblasts/cancer-associated fibroblasts are a pivotal component in the TAFs/CAFs-rich stroma contributing to tumorigenesis. Accumulating evidences indicate targeting those fibroblasts may facilitate anti-cancer therapy by affecting oncogenic interactions between cancer cells and the assorted cell types constituting the tumor microenvironment. Intriguingly, Hsp90 inhibitors suppressed the reactive stroma phenotype

in hepatic stellate cells and induced caspase-8-mediated apoptosis via sphingomyelinase- and NF-κB-dependent pathways (Myung *et al.,* 2009).

In addition, MAPK signaling pathways was activated in pancreatic stellate cells and colon cancer through upregulation of periostin, a component of extracellular matrix to promote tumor metastasis and invasion, suggesting targeting Hsp90-client oncogenic signalings may represent a novel approach for anti-stroma regimen in the future (Erkan *et al*., 2007; Bao *et al*., 2004).

**Small molecule inhibitors and their role in cancer treatment**

Almost two decades ago, Whitesell *et al.* firstly demonstrated Hsp90-involved heteroprotein complex formation was required for v-src-mediated morphologic transformation and benzoquinone ansamycins such as Geldanamycin (GA), can inhibit Hsp90-src through competitive-binding to Hsp90 (Whitesell *et al*., 1994).

Recently, Roué *et al.* (2011) reported the Hsp90 inhibitor retaspimycin hydrochloride restored drug sensitivity in proteasome inhibitor bortezomib-resistant aggressive mantle cell lymphoma (MCL). In their study, one of Hsp70 family members, BiP/GRP78 was up-regulated in aggressive B-cell malignancies including MCL and was responsible for constitutive or induced-bortezomib resistance. Retaspimycin hydrochloride in combination with bortezomib dissociated Hsp90-BiP/GRP78 complex, causing the latter to be depleted thus affecting the UPR and restoring apoptosis (Roué *et al.,* 2011). These findings added a novel function of Hsp90 in cancer treatment by inhibition of UPR-related oncogenic phenotypes including drug resistance and evasion of apoptosis. With the help of X-ray crystallography and structure-based drug design to improve potency, a second class of synthetic Hsp90 inhibitors have been reported, Novartis is a novel Hsp90 inhibitor and has the highest affinity for the $NH_2$-terminal ATP-binding site among synthetic small molecule inhibitors. Effects of Novartis include inhibition and/or repression of tumor growth in tumor xenografts with a variety of types of human cancers, blockage of tumor cell invasion and metastasis both *in vitro* and *in vivo*, and depletion of client proteins including BCR-ABL, PKB and HIF-α (Eccles *et al*., 2008).

Recent studies show impressive synergistic action of Novartis with melphalan, doxorubicin, and suberoylanilide hydroxamic acid (SAHA) in multiple myeloma and build the experimental foundation for clinical trials (Kaiser *et al*., 2010). Of notice, cell surface Hsp90 was found on melanoma cells, fibrosarcoma cells, bladder cancer cells, prostate cancer cells as well as neuronal cells and play an important role to control cancer cell migration independent of intracellular Hsp90 pool function. Intriguingly, extracellular Hsp90α once hyperacetylated by HDAC inhibitor acted as a chaperone for MMP-2 to promote tumor cell invasion, suggesting inhibition of extracellular hyperacetylated Hsp90α may affect tumor invasion and metastasis (Yang, 2008).

**Table** 2. Hsp90 inhibtors in clinical trials

| Hsp90 inhibitor | Clinical Trial Phase | Cancer |
|---|---|---|
| Geldanamycin | I* | Thyroid (hepatotoxicity *in vivo* |
| 17-AAG (Tanespimycin) | II/III | Breast, leukemia, prostate |
| 17-DMAG | I | Breast, leukemia |
| Retaspimycin hydrochloride | I/II/III | pancreatic, MCL |
| Radicicol | I | *In vitro*( no + *in vivo* results) |
| Purine-scaffold inhibitors | I | Hodgkin's lymphoma, CLL |
| Shepherin | - | Leukemia (animal model) |
| Pyrazoles | - | Prostate cancer (*in vitro*) |
| Mesylate | I | HT-29 model |
| Novartis | - | Breast, prostate |
| Oxime derivative | - | Breast (*in vitro*) |
| Novobiocin | II | Breast and melanoma (*in vitro*) |
| Coumermycin A1 | | Breast (*in vitro*) |
| Cisplatin | I/II/III/IV | Head and neck |
| Vorinostat/SAHA (HDAC6) | I/II | Leukemia, head and neck |
| Romidepsin | II | T-cell lymphoma |

*Clinical Trial suspended

**Source**: (Li, 2011).

In order to survive under the harsh conditions within the tumor microenvironment, cancer cells typically become dependent on stress-inducible HSPs to become refractory to chemotherapy, tolerant to hypoxia, resistant to apoptosis, and to suppress antitumor immunity, all the while acquiring the properties of invasiveness and metastasis during cancer progression. To date, more than 200 HSP client proteins have been identified involving nearly all fundamental cellular activities and processes, including cell growth, proliferation, and cell survival (Jego *et al*., 2010).

Interestingly, many cancer-associated proteins have been reported as HSP clients, likely as a mechanism for promoting oncogenic transformation. Therefore, targeting HSPs would result in simultaneous inhibition of

multiple signaling pathways responsible for modulation of various events involved in cancer progression for a broad range of tumor types, such as neoplastic growth, sustained angiogenesis, chemotherapeutic resistance, evasion of cell death, and ultimately, invasion and metastasis (Barginear *et al*., 2008). Although the exact molecular mechanism(s) of HSP inhibitors have not yet been fully determined, a significant number of client proteins are either part of mechanistic studies (bench) or under evaluation as part of clinical trials (bedside). For example, histone deacetylase complex (HDAC) inhibitors as novel anticancer agents are found to hyperacetylate Hsp90, causing an increase in its binding to an Hsp90 inhibitor, ultimately showing anti-tumor activity in leukemia and prostate cancer (Barginear *et al*., 2008).

Glucose-regulated protein 78 kDa (GRP78), also known as immunoglobulin heavy chain binding protein (BiP), is a member of the HSP family of molecular chaperones and serves as an unfolded protein response marker. GRP78 is involved in cellular adaptation and survival to facilitate tumorigenesis through active interaction with a variety of partners/ligands within tumor cells (Dudek *et al*., 2009). On the basis of shared homology with other HSP family proteins, GRP78 is also a molecular target of HDAC inhibitors, resulting in the phosphorylation and activation of initiating factor 2α and an increase in ATF4 and C/EBP homologous protein synthesis, all of which are important for progression of certain cancer types (Kahali *et al*., 2010).

Thus, overall, HSPs are implicated in cancer and have been shown to specifically interfere with current antitumor therapies that target phenotypic responses like apoptosis, necrosis, autophagy, and senescence. Not surprisingly, inhibition of Hsp90's ATPase activity and disruption of ongoing chaperone folding cycles results in dissociation, destabilization, and proteasomal degradation of a variety of client proteins, including the cancer-associated targets Bcl-2, Apaf-1, PKB, and MMP-2 (Jego *et al*., 2010; Wang *et al*., 2009).

Even though clinical trials often show inherent toxicity of Hsp90 inhibitors and strong induction of cytoprotective function of Hsp70, a combination of Hsp90 and Hsp70 inhibitors with traditional chemo- and/or radio-therapy may provoke tumor regression in a synergistic manner(Jego *et al*., 2010; Wang *et al*., 2009).

## CONCLUSION AND RECOMMENDATIONS

Cancer is a threat both for human and animal life and is, thus, one of the least understood diseases having no effective therapy. It expresses antigens that are recognized as foreign by the immune system of tumor-bearing hosts. In order to survive under the harsh conditions within the tumor microenvironment, cancer cells typically become dependent on stress-inducible HSPs to become refractory to chemotherapy, tolerant to hypoxia, resistant to apoptosis, and to suppress antitumor immunity, all the while acquiring the properties of invasiveness and metastasis during cancer progression. Interestingly, many cancer-associated proteins have been reported as HSP clients, likely as a mechanism for promoting oncogenic transformation. Therefore, targeting HSPs would result in simultaneous inhibition of multiple signaling pathways responsible for modulation of various events involved in cancer progression for a broad range of tumor types, such as neoplastic growth, sustained angiogenesis, chemotherapeutic resistance, evasion of cell death, and ultimately, invasion and metastasis. Not surprisingly, inhibition of HSPs activity and disruption of ongoing chaperone folding cycles results in dissociation, destabilization, and proteasomal degradation of a variety of client proteins, including the cancer-associated targets Bcl-2, Apaf-1, PKB, and MMP-2.

Based on the above conclusive statements, the following points are recommended:

- ✓ Inhibiting Hsp90 in combination with other heat shock proteins, such as Hsp70 and Hsp27, which may be an alternative strategy to enhance synergistic cancer therapy with minimum off-target side effect.
- ✓ Additional studies should be accomplished to define the precise molecular mechanism(s) of HSPs which will ultimately allow for the identification of reliable biomarkers for monitoring the effects of HSPs suppression *in vivo*.
- ✓ Accumulating evidences suggest alternative locations of HSPs, especially Hsp90 inside or outside of the cells, which indicates a unique role as part of a tumor-specific phenotype. Therefore, the use of specific cellular or subcellular targeting for chaperones needs to be considered and developed.

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