Published in final edited form as:

Future Oncol. 2009 June ; 5(5): 679–688. doi:10.2217/fon.09.30.

Impact of heat-shock protein 90 on cancer metastasis

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Abstract

Cancer metastasis is the result of complex processes, including alteration of cell adhesion/motility in the microenvironment and neoangiogenesis, that are necessary to support cancer growth in tissues distant from the primary tumor. The molecular chaperone heat-shock protein 90 (Hsp90), also termed the ‘cancer chaperone’, plays a crucial role in maintaining the stability and activity of numerous signaling proteins involved in these processes. Small-molecule Hsp90 inhibitors display anticancer activity both *in vitro* and *in vivo*, and multiple Phase II and Phase III clinical trials of several structurally distinct Hsp90 inhibitors are currently underway. In this review, we will highlight the importance of Hsp90 in cancer metastasis and the therapeutic potential of Hsp90 inhibitors as antimetastasis drugs.

Keywords

17AAG; 17DMAG; cell adhesion; cell motility; geldanamycin; Hsp90; metastasis; molecular chaperone; neoangiogenesis

Cancer is the leading cause of death worldwide, and was responsible for 7.9 million deaths in 2007 [201]. However, the major cause of human cancer deaths is metastasis to distant tissues, not growth of the primary tumor itself. Cancer metastasis is the result of a series of sequential and highly orchestrated processes, including alteration of cancer cell adhesion/motility and the ability to induce neoangiogenesis. Cancer cells must rely on a number of signaling proteins that regulate these events [1,2]. Many of these signaling proteins are ‘clients’ of heat-shock protein 90 (Hsp90), in that they rely on this molecular chaperone for proper folding, stability and function (FIGURE 1 & TABLE 1).

Heat-shock protein 90

Heat-shock protein 90 is an abundant molecular chaperone that is further overexpressed or activated in cancer cells, suggesting that it could be a crucial regulator of growth and/or survival of tumor cells [3,4]. Hsp90 association is important for maintaining the stability and function

of numerous proteins referred to as client proteins [5]. Hsp90 clients are frequently mutated or activated in cancer cells, and include the oncogenic tyrosine kinase v-Src, the mutated oncogene Bcr/Abl, the receptor tyrosine kinases ErbB2 and c-MET, and the serine/threonine kinase Raf-1 [5]. Client—protein interaction is regulated by the adenine nucleotide binding status of Hsp90. Nucleotide exchange and ATP hydrolysis that occur in an amino-terminal binding pocket in Hsp90 direct the mechanistic binding, chaperoning and release of client protein in what is referred to as the chaperone cycle [6,7]. Hsp90 bound to ATP has higher affinity for client proteins than its ADP-binding counterpart. Although Hsp90 itself has weak ATPase activity, the ATPase activity is both positively and negatively regulated by cochaperones, such as Aha1, p60^{Hop}, p50^{cdc37} and p23 [7]. Disruption of the association of client proteins with Hsp90 is generally followed by their ubiquitination and subsequent degradation in proteasomes [8,9]. Hsp90 inhibitors block cancer cell proliferation *in vitro* and cancer growth *in vivo* [9]. These drugs inhibit Hsp90 function by competing with ATP binding, thereby freezing the chaperone cycle, which in turn decreases the affinity of Hsp90 for client proteins and leads to proteasome-mediated client protein degradation (FIGURE 2) [10-12]. Several structurally distinct Hsp90 inhibitors are currently being evaluated for anticancer activity in numerous Phase II and several Phase III clinical trials, both as single agents and in combination with other cancer drugs [8,13]. In this review we will examine the role of Hsp90 in metastasis-related events, including cell adhesion, cell motility and angiogenesis, and we will highlight the therapeutic possibility of using Hsp90 inhibitors to impede cancer metastasis.

Hsp90 & cell adhesion

Cell adhesion to extracellular matrix (ECM) proteins modulates metastasis through a variety of signals important for mitogenesis and motogenesis [14]. Alteration of this adhesion is one of the earliest in the cascade of events that make cancer metastasis possible [14]. Integrins, dimeric transmembrane adhesion receptors comprised of α - and β -subunits, are key modulators of cell adhesion at sites of contact with ECM. Ligation of ECM proteins to integrins through the large integrin ectodomain modulates signaling via the cytosolic kinases that bind the short integrin cytoplasmic tail [14,15].

Focal-adhesion kinase (FAK) is a cytosolic nonreceptor tyrosine kinase that was originally identified as a substrate of the v-Src onco-gene [16]. FAK resides at sites of integrin clustering and interacts with integrin cytoplasmic tails. This binding stimulates the recruitment of other adaptor proteins, such as p130^{Cas}, paxillin and talin, to form complexes known as focal adhesions [16]. At sites of integrin-mediated adhesion, these macromolecular assemblies function not only as sites of mechanical linkage to the ECM, but also serve as scaffolds for the numerous signaling proteins that are necessary to facilitate the dynamic process of cell-adhesion formation and their eventual turnover [16]. The function of FAK is regulated by phosphorylation, particularly tyrosine phosphorylation [17]. The prototypical Hsp90 inhibitor geldanamycin (GA) stimulates the proteasome-mediated degradation of FAK in many types of cancer cells and markedly reduces the half-life of newly synthesized FAK protein without altering the level of FAK mRNA [18]. Furthermore, GA reduces tyrosine phosphorylation of FAK and assembly of focal adhesions, and inhibits FAK-dependent subsequent events, including rearrangement of the actin cytoskeleton, cell migration and cell invasion [19-21].

Another key player in the cell-adhesion process is integrin-linked kinase (ILK). ILK is a serine/threonine protein kinase containing four ankyrin-like repeats that has been identified as an interacting partner of the β 1-, β 2- and β 3-integrin cytoplasmic tails [22]. ILK also complexes with PINCH1 and PINCH2, paxillin, α -parvin and β -parvin, and these associations are reported to be important for cell polarization and adhesion [22]. ILK is known to interact with Hsp90, together with the co-chaperone p50^{cdc37} through amino acid residues 377–406 of the kinase. Hsp90 inhibitors induce proteasomal degradation of ILK in several types of normal and cancer

cells, thereby impeding rearrangement of the actin cytoskeleton and cell adhesion [23]. These data indicate that Hsp90 is an important modulator of the signaling pathways required for integrin-mediated cell adhesion.

Hsp90 & cell motility

Increased cell motility is a typical feature of metastatic cancer cells. In order for migration to occur, a defined sequence of morphological changes must take place. Such cellular locomotion is regulated by a variety of environmental signals and is largely dependent upon the dynamic rearrangement of the cytoskeletal protein, actin [24]. During this process, actin is recruited to, and forms a complex with, focal adhesions. Depolymerization and repolymerization of the actin cytoskeleton at the site of leading edge adhesion provides the moving force for cell motility [24]. Concomitantly, the processes of cytosolic translocation and detachment of the lagging edge requires the production of matrix-degrading proteases to degrade the underlying ECM proteins. Remodeling of ECM proteins is a key step for cellular invasion to surrounding tissues and penetration into the basement membrane [25].

Receptor tyrosine kinases (RTKs) are the major group of Hsp90 client proteins implicated in the process of cell motility [26]. In the presence of chemo-attractants, RTKs are activated and complex with intracellular signaling molecules, stimulating cell motility via classical signal transduction pathways [26]. The avian erythroblastosis oncogene B (*ErbB*) family of RTKs has been found to consistently play a key role in cancer progression and metastasis [27]. ErbB2 (also called HER2/neu) is a member of this protein family. ErbB2 amplification is often observed in breast and ovarian cancers [28,29], and its expression level correlates with increased metastatic activity and poor prognosis [29,30]. ErbB2 is unique among the ErbB family of proteins in that it is exquisitely sensitive to Hsp90 inhibition [31]. Hsp90 has been shown to interact with ErbB2 through the ErbB2 kinase domain [32]. GA induces the dissociation of this complex, after which rapid proteasome-mediated degradation of ErbB2 takes place [33,34], as does inhibition of the maturation of newly synthesized ErbB2 [35]. By contrast, another ErbB family protein, EGF receptor (EGFR; also called ErbB1), is resistant to GA-induced degradation, although its amino acid sequence is highly homologous to that of ErbB2. Mutation of EGFR can result in acquired sensitivity of the mature protein to Hsp90 inhibitors. It was first reported in non-small-cell lung cancer cells that point mutation of residues L858 and T790 or deletion of amino acids E746—A750 makes the EGFR protein sensitive to GA-induced degradation [36]. It was later reported that insertion mutations in exon 20 generate the same effect [37], suggesting that somatic mutations in the kinase domain modulate the Hsp90 dependency of EGFR. Since the positively charged hydrophobic region in the kinase domain of ErbB2 has been shown to be important for its association with Hsp90, those mutations may affect the conformation of the kinase domain of EGFR, rendering it vulnerable to Hsp90 inhibitors [38].

Hepatocyte growth factor (HGF) is the one of the best characterized growth factors involved in cell motility. Also known as scatter factor, HGF is known to be a potent mitogen, motogen and morphogen that acts primarily upon epithelial and endothelial cells and hematopoietic progenitor cells. HGF regulates such activities by activating a tyrosine kinase signaling cascade after binding to the proto-oncogenic RTK cellular-MET (c-MET). MET is activated in cancer cells through ligand-dependent autocrine or paracrine mechanisms, and its expression is upregulated in many types of cancer cells and correlates with higher cell motility [39]. GA destabilizes both mature and immature MET in breast and small cell lung cancer cells [21, 40]. GA also disrupts HGF-induced association of MET with FAK and inhibits subsequent rearrangement of the actin cytoskeleton and cell motility [21]. Furthermore, GA has been shown to inhibit HGF-induced cell scattering and MET-dependent transformation [41].

The type 1 insulin-like growth factor receptor (IGF-1R) is an RTK that is also important for cell motility and metastasis [42]. IGF-1R expression is upregulated in many types of cancer cells [43,44], correlating with cancer progression [45]. Hsp90 inhibitors both reduce IGF-1R expression at the transcriptional level and induce IGF-1R protein degradation [44,46,47]. The GA analog 17-AAG has been shown to inhibit IGF-1-induced phosphorylation of IGF-1R and IGF-1R-induced cell migration in pancreatic cancer cells [47]. Furthermore, 17-AAG has been reported to inhibit tumor growth *in vivo* through downregulation of IGF-1R and inhibition of IGF-1R-mediated downstream signals [47].

Signaling molecules downstream of RTKs are also regulated by Hsp90. c-Src is a nonreceptor tyrosine kinase implicated in a variety of RTK-mediated signaling pathways related to cell motility [48]. It is rarely mutated but is frequently overexpressed in many types of cancer cells [49]. c-Src physically associates with RTKs and is transiently activated by multiple growth factors or ECM proteins which, in turn, activates signaling molecules further downstream [49]. Hsp90 has been reported to interact with c-Src and to be important for its maturation [50,51]. Hsp90 also seems to play a unique and seemingly paradoxical role in c-Src activity. Disruption of the c-Src—Hsp90 interaction by GA ultimately induces Src downregulation, which, in turn, attenuates subsequent signaling pathways [52]. However, on a shorter time scale, dissociation from Hsp90 results in transient upregulation of c-Src and c-Src-coupled ErbB2, which, in turn, stimulates downstream signaling molecules such as Akt [52,53]. Indeed, 17-AAG has been reported to enhance the incidence of breast cancer bone metastases indirectly by promoting osteolytic lesions through activation of osteoclastogenesis, a process that is very dependent on, and sensitive to, activated c-Src, even though the drug inhibited primary tumor growth in mammary fat pads of athymic mice [54]. Subsequently, 17-AAG was reported to promote osteoclast differentiation through integrin β 3-mediated c-Src and Akt activation, and to indirectly promote prostate cancer growth in bone, while inhibiting subcutaneous growth of the primary tumor [55]. These findings suggest that Hsp90 can have both a negative and a positive influence on c-Src activity.

The serine/threonine kinase phosphoinositide 3-kinase (PI3K) also interacts with RTKs, and has been shown to be linked to the process of cell motility. PI3K exists as heterodimers composed of catalytic and regulatory subunits [56]. Once PI3K is activated by interaction with an activated intracellular domain of an RTK, it phosphorylates its substrate phosphatidylinositol (4,5) biphosphate (PIP2) and converts it to phosphatidylinositol (3,4,5) triphosphate (PIP3). PIP3 induces phosphorylation and activation of the serine/threonine kinase Akt through 3-phosphoinositide-dependent protein kinase (PDK)-1 and -2 [57], and activated Akt then modulates numerous downstream signaling molecules that are important for cell motility [58]. Hsp90 plays an essential role in maintaining the integrity of this signaling pathway. PDK1 and Hsp90 associate through the PDK1 kinase domain [59]. GA disrupts this interaction, inducing degradation of PDK1 and reducing phosphorylation of Akt [59], which, along with the co-chaperone p50^{cdc37}, also directly associates with Hsp90 [60]. Hsp90 inhibition reduces phosphorylation of Akt and induces its ubiquitination and subsequent proteasomal degradation without any effect on the PI3K protein level [59,61,62].

The rho GTPases Rho, Rac and Cdc42 act as a molecular switch for the RTK-mediated rearrangement of the actin cytoskeleton [63]. When coupled with RTKs, many signaling proteins such as c-Src and PI3K can modulate Rho GTPase activity [64-66]. Taken individually, Rho, Rac and Cdc42 induce different morphological changes within the actin cytoskeleton, namely stress-fiber, lamellipodia and filopodia formation, respectively. Cdc42 is known to associate with activated Cdc42-associated kinase 1 and 2 (Ack1 and Ack2), members of the FAK family of nonreceptor tyrosine kinases [67,68]. Ack1 induces phosphorylation of p130^{Cas} and recruits the adaptor protein Crk, stimulating subsequent rearrangement of the actin cytoskeleton [67]. Hsp90 was identified as an Ack1-interacting

protein by biochemical affinity purification [69]. GA reduces the kinase activity of Ack1 without any effect on total Ack1 protein level, and suppresses Ack1-induced tumorigenesis in an animal model [69]. Hsp90 also serves as a regulatory component in Ack2 function, as it is required for both the binding of Ack2 to Cdc42, and for Ack2 tyrosine kinase activity. This association occurs through the Ack2 kinase domain and it can be disrupted by GA, thereby inhibiting Ack2 kinase activity and again supporting the role of Hsp90 in the regulation of Cdc42 signaling [68]. Taken together, these data support the notion that Hsp90 plays a crucial role in RTK-mediated rearrangement of actin cytoskeleton from the first events through to propagation of downstream signals.

Matrix remodeling through matrix-degrading enzymes, such as plasminogen activators and matrix metalloproteinases (MMPs), is a crucial event for cell invasiveness [25]. The level of MMPs is increased in many types of cancer cells and their expression correlates strongly with metastasis [70-72]. GA has been shown to attenuate HGF-induced urokinase (uPA) activity, and subsequent activation of MMP-2 and MMP-9 [41]. Furthermore, GA has been reported to inhibit chemical hypoxia-induced activation of uPA, MMP-2 and MMP-9 [21]. Hyaluronic acid is a major component of the ECM. Hyaluronic acid has the capacity to stimulate MMP-9 expression and secretion through FAK- and nuclear factor (NF)- κ B-mediated signaling pathways [73]. 17-AAG reduces both phosphorylation of FAK and NF- κ B activation, leading to inhibition of MMP-9 activity and reduction of subsequent cell invasion into the ECM [73]. Together, these data suggest that Hsp90 modulates protease-dependent matrix remodeling.

Hsp90 & neoangiogenesis

Neoangiogenesis is critical for providing blood flow to growing cancer metastases [74]. This process involves the growth of new blood vessels and capillary formation from the pre-existing vasculature that is induced by various angiogenic growth factors [74]. Hypoxia-inducible factor (HIF)-1 is a transcriptional activator that regulates the transcription of a variety of proangiogenic genes including *VEGF* [75]. Although HIF-1 protein is normally rapidly degraded by the proteasome subsequent to ubiquitination by the oxygen-dependent E3 ubiquitin ligase pVHL, HIF-1 is stabilized and activated by hypoxia [75]. Thus, HIF-1 levels are frequently elevated in tumor cells due to their hypoxic environment or by mutation/loss of the *VHL* gene, which, in turn, stimulates pro-angiogenic signaling [76]. Hsp90 has been shown to interact with HIF-1 through the Per-ARNT-Sim domain of HIF-1 [77], and disruption of this interaction by GA treatment induces ubiquitination and proteasome-mediated degradation of the HIF-1 protein [78]. Consequently, GA also inhibits HIF-1-mediated transcription of downstream signaling proteins, and thus hypoxia-induced angiogenesis [79].

The VEGF receptor (VEGFR) family belongs to a superfamily of RTKs. Its members are key mediators of angiogenesis and are specifically expressed in endothelial cells [80]. VEGF ligand regulates VEGFR activity and it is produced by cancer cells and surrounding fibroblasts [80]. Activation of VEGFR stimulates endothelial cell proliferation and migration, and increases the permeability of the vascular endothelial layer through tyrosine kinase signaling [80]. Hsp90 is known to bind to the c-terminal tail of VEGFR2, a VEGFR family member [81]. Hsp90 inhibitors attenuate the expression of VEGFR and VEGFR-dependent tyrosine phosphorylation of FAK, thereby inhibiting subsequent signaling pathways [19,20,81]. Furthermore, Hsp90 inhibitors have been shown to inhibit VEGF secretion and VEGFR expression. In doing so, subsequent endothelial cell proliferation, migration, tubular differentiation and cord formation are impeded, and endothelial cell apoptosis is induced [21, 82,83].

VEGF family members are structurally related to the PDGFs, another family of key mediators of the angiogenic process [84]. The PDGF receptor (PDGFR) is frequently overexpressed and

activated in cancer cells, and its expression level is correlated with metastatic activity [84, 85]. In cancer cells, Hsp90 has been shown to interact with PDGFR α together with the co-chaperone p50^{cdc37} [86]. Interestingly, inhibition of Hsp90 induces ubiquitination and proteasome-mediated degradation of PDGFR α in cancer cells but not in normal cells [86]. Taken together, these data suggest that Hsp90 is an important regulator of neoangiogenesis from the transcriptional level to the protein level, and may prove to be a potent and selective anticancer target for anti-angiogenic therapy.

Extracellular Hsp90 & metastasis

Recent studies indicate that Hsp90 expression is not confined to the cytosol; in fact, it has been shown that many types of cells express Hsp90 on the cell surface and secrete Hsp90 into the extracellular space [87]. Moreover, the level of cell surface Hsp90 has been shown to increase in cancer cells and correlates with metastatic activity [88]. Although the mechanism is not clear, Hsp90 secretion is known to be stimulated by environmental stresses and growth factors [89,90], and affected by post-translational modification, such as phosphorylation and acetylation [91,92]. Since Hsp90 does not have a conventional secretion signal sequence, Hsp90 is probably secreted by an unconventional secretion pathway [93]. Indeed, Hsp90 is known to exist in exosomes and it has been demonstrated that at least a portion of Hsp90 secretion occurs via the exosomal pathway [89,94]. Extracellular Hsp90 may play a role in cancer metastasis distinct from, but perhaps overlapping with, its intracellular chaperone function. It was originally shown to have a role in cell motility by a functional protein screening assay for the invasion of fibrosarcoma cells [95]. Hsp90 exists on the cell surface together with MMP2 and inhibition of extracellular Hsp90 with either a neutralizing antibody or cell-impermeable small-molecule Hsp90 inhibitor decreases MMP2 activity and cell invasion [95]. Around the same time as the original study, another group identified extracellular Hsp90 to be involved in the migration processes of the developing nervous system [96]. Hsp90 is expressed on the neuronal cell surface and treatment with neutralizing antibody against extracellular Hsp90 decreased reorganization of the actin cytoskeleton and, thus, cell migration [96]. Subsequent studies using inhibitors against extracellular Hsp90 have revealed antimigratory activity in bladder cancer, melanoma, prostate cancer, breast cancer and dermal cells [89,91,97]. A neutralizing antibody against extracellular Hsp90 has been shown to inhibit melanoma metastasis *in vivo* and to result in prolonged survival in murine xenograft model systems [97,98].

Although little is known regarding its mechanism of action, extracellular Hsp90 seems to interact with cell surface receptors. Hsp90 has been shown to stimulate cell migration through the cell surface receptor CD91, independent of its ATP-binding and ATPase activity [89]. In addition, it has been reported that Hsp90 binds to the extracellular domain of ErbB2 [99]. Neutralizing antibody against extra cellular Hsp90 attenuates heregulin-induced ErbB2 phosphorylation, downstream kinase signaling, rearrangement of the actin cytoskeleton and subsequent cell migration [99]. Furthermore, a cell-impermeable small-molecule Hsp90 inhibitor inhibits focal adhesion complex formation and rearrangement of the actin cytoskeleton, and subsequent cell migration and invasion [97]. These data suggest that extracellular Hsp90 might play an important role in cell motility and metastasis.

Conclusion

This review supports the concept that Hsp90 is an attractive target for antimetastatic therapy as it is implicated in multiple aspects of the metastatic process, most notably cell adhesion, cell motility and neoangiogenesis. For the sake of simplicity and clarity, we have reviewed the literature categorically by process, and we summarized the role of Hsp90 in each process separately. However, because metastatic signaling pathways dependent on Hsp90 overlap, we

fully expect the role of Hsp90 in metastasis to be more complex, and we suggest that inhibition of Hsp90 is likely to be more potent than would be predicted for an inhibitor of only one aspect of metastasis.

Although Hsp90 inhibitors are well known to have anticancer activity *in vitro* and *in vivo*, and their therapeutic potential has been investigated in many clinical trials, less attention has been paid to their antimetastatic activity. In fact, only recently have Hsp90 inhibitors been shown to display promising antimetastatic activity in a broad range of cancer cells. Further investigation, especially utilizing models of *in vivo* metastasis, is required to unveil the complete mechanism of Hsp90-organized cancer metastasis. It is important to note, however, that reports have been published showing that Hsp90 inhibitors can indirectly accelerate bone metastasis through the activation of osteoclastogenesis in two distinct experimental murine bone metastasis models [54,55]. It is not clear whether Hsp90-dependent acceleration of bone metastasis also occurs in humans, but it would be prudent as a facet of the ongoing clinical evaluation of these agents to closely monitor the potential impact of Hsp90 inhibitors on bone density and the bone microenvironment.

Future perspective

Hsp90 inhibitors have entered Phase II and III clinical trials, and have shown therapeutic activity in several types of cancer. Many novel small-molecule Hsp90 inhibitors are currently being assessed *in vitro*, in animal models and in clinical trials [100-103]. In addition, non-small-molecule Hsp90 inhibitors, including the Hsp90 antibody Mycograb® and the peptide inhibitor shepherdin, have displayed anticancer activity in preliminary studies [104]. It is probable that further investigation will identify Hsp90 inhibitors with improved therapeutic activity and better pharmacologic properties.

Executive summary

Heat-shock protein 90

- Heat-shock protein 90 (Hsp90) is a molecular chaperone that is important for the maturation, stability and activity of numerous cancer-related proteins.
- Hsp90 is frequently overexpressed and activated in cancer cells.
- Hsp90 inhibitors have entered Phase III clinical trials.

Intracellular Hsp90 & cancer metastasis

- Hsp90 inhibitors display strong antimetastatic activity in many types of cancer cells.
- Cell adhesion is regulated by Hsp90 through cell matrix adhesion-related kinase activity.
- Cell motility is regulated by Hsp90 impact on the multiple steps of receptor tyrosine kinase-mediated signaling.
- Neoangiogenesis is regulated by Hsp90 from the transcriptional to the protein level.
- The antimetastatic activity of Hsp90 inhibitors has to be better defined in experimental *in vivo* spontaneous metastasis models.

Extracellular Hsp90 & cancer metastasis

- Hsp90 exists not only in the intracellular space, but also in the extracellular space.

- Although the extracellular pool of Hsp90 is increased in many types of cancer cells, the mechanism of increase is not clear.
- Inhibitors of extracellular Hsp90 display antimetastatic activity both *in vitro* and *in vivo*.
- Although extracellular Hsp90 seems to regulate cell motility through matrix-degrading proteases and cell surface receptors, further investigation is needed to better define its complete mechanism(s) of action.

Hsp90 inhibitors have been shown to display promising therapeutic activity when used in conjunction with proteasome inhibitors or kinase inhibitors [105,106]. In a preclinical study, the Src and Bcr-Abl kinase inhibitor dasatinib was also shown to reverse Hsp90 inhibitor-dependent cancer cell proliferation in bone [55]. Future experimentation is needed to discern which combination therapies will prove to be most beneficial, as the possibilities are vast.

Although extracellular Hsp90 is known to contribute to cancer metastasis, the molecular mechanisms underlying its secretion and subsequent actions are not clear. Further investigation is warranted to verify the molecular determinants of Hsp90 secretion. It is possible that extracellular Hsp90 may have nearly as varied a list of client proteins as does its intracellular counterpart, thereby contributing to its diverse effects on the metastatic process.

Acknowledgments

Financial & competing interests disclosure The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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Figure 1. Heat-shock protein 90 plays multiple roles in cancer metastasis

EGFR: EGF receptor; HIF: Hypoxia-inducible factor; Hsp90: Heat-shock protein 90; IGF-1R: Type 1 IGF receptor; MMPs: Matrix metalloproteinases; PDGFR: PDGF receptor; VEGFR: VEGF receptor.

Figure 2. Hsp90 inhibitors induce client protein degradation and/or inactivation
GA: Geldanamycin; Hsp90: Heat-shock protein 90.

Table 1

A partial list of heat-shock protein 90-regulated signaling proteins involved in various aspects of cancer metastasis.

Protein	Description	Role of Hsp90	Ref.
<i>Cell adhesion</i>			
FAK	Nonreceptor tyrosine kinase	Stability/activity	[16-19]
ILK	Serine/threonine kinase	Stability	[21]
<i>Cell motility</i>			
ErbB2 (HER2/neu)	Receptor tyrosine kinase	Stability/maturation	[29-33]
EGFR (mutant)	Receptor tyrosine kinase	Stability/maturation	[34-36]
MET	Receptor tyrosine kinase	Stability/activity	[19,38,39]
IGF-1R	Receptor tyrosine kinase	Stability/activity	[42,44,45]
c-Src	Nonreceptor tyrosine kinase	Maturation/activity	[48-53]
PDK1	Serine/threonine kinase	Stability	[57]
Akt	Serine/threonine kinase	Stability/activity	[57-60]
Ack1/2	Nonreceptor tyrosine kinase	Activity	[66,67]
uPA	Serine protease	Activity	[19,39,71]
MMP-2/9	Serine protease	Expression/secretion	[39,89,93]
<i>Neoangiogenesis</i>			
HIF-1	Transcriptional activator	Activity/stability	[75-77]
VEGFR	Receptor tyrosine kinase	Activity	[17-19,79-81]
PDGFR	Receptor tyrosine kinase	Stability	[84]

FAK: Focal adhesion kinase; HIF: Hypoxia-inducible factor; Hsp: Heat-shock protein; IGF-1R: Type 1 insulin-like growth factor receptor; ILK: Integrin-linked kinase; MMP: Matrix metalloproteinase; PDGFR: PDGF receptor; VEGFR: VEGF receptor.