Review

Heat-shock Protein 90 (Hsp90) as a Molecular Target for Therapy of Gastrointestinal Cancer

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Abstract. *Anticancer drug development strategies critically involve the identification of novel molecular targets which are crucial for tumorigenesis and metastasis. In this context, the molecular chaperone heat-shock protein 90 (Hsp90) has gained interest as a promising anticancer drug target, due to its importance in maintaining the stability, integrity, conformation and function of key oncogenic proteins. These Hsp90 "client proteins" have been demonstrated to play fundamental roles in the processes of signal transduction, cell proliferation and survival, cell cycle progression and apoptosis, as well as other features of malignant cells, such as invasion, tumor angiogenesis and metastasis. The cancer selectivity and antitumoral effects of Hsp90 inhibitors are mediated by simultaneous and combined actions, in terms of directly affecting multiple cancer targets and pathways. Several Hsp90 inhibitors, including the geldanamycin derivative 17-allylamino-17-demethoxygeldanamycin (17AAG), have displayed convincing antineoplastic efficacy and cancer selectivity in a variety of preclinical models, including gastrointestinal carcinomas. Importantly, some Hsp90 inhibitors have now progressed to phase I/II clinical testing. Against this background, the following review focuses on the current preclinical experience and value of targeting Hsp90 for the therapy of gastrointestinal carcinomas.*

The heat-shock protein 90 (Hsp90) belongs to a family of molecular chaperones which are of central importance for the sophisticated process of maintaining intracellular protein homeostasis. In particular, Hsp90 is involved in regulating the cell function of both non-malignant and malignant cell types, comprising the processes of *de novo* protein folding during protein synthesis, translocation of proteins across membranes, quality control in the endoplasmatic reticulum, proteolytic turnover of important mediators of cell growth, cell differentiation and cell survival (1, 2). Furthermore, chaperones participate in the post-translational regulation of signaling molecules, (dis)/assembly of transcriptional complexes, and in the processing of immunogenic peptides by the immune system (3). Thus the number of proteins known to interact with Hsp90 (commonly referred to as "Hsp90 client proteins") is expanding rapidly. Importantly, chaperones, including Hsp90, are ubiquitously expressed, even under constitutive non-stressed conditions (*i.e.* Hsp90 comprises 2% of total cytosolic protein). However, following environment-mediated cell stress with consecutive protein damage, for instance, chaperoning functions are needed and the expression of Hsps is substantially up-regulated (4).

Hsp90 and cancer. The chaperone Hsp90 has recently been linked to diseases of exceptional importance, including cerebro- and cardiovascular diseases (ischemia and reperfusion) (5), the process of infection (6), autoimmune diseases (7) and cancer. Regarding the latter, cancer cells experience various types of stress in their unfavorable environment, comprising acidosis, hypoxia, high interstitial pressure and nutrient deprivation (8). As a consequence, Hsp90 can frequently be found to be up-regulated in tumor cells, in transformed cells (9), malignancies of the hematopoietic system (10) and in solid tumors of various entities including melanoma (11), ovarian and endometrial carcinomas (12, 13), breast cancer (14), as well as in gastric and pancreatic carcinomas (15-17). Moreover, recent data have proven an essential role of Hsp90 in facilitating malignant transformation and thus being critical for the development of solid malignancies. Importantly, Hsp90

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activity in cancer cells seems to be closely related to the overall proliferative potential of these malignant cells, and has been shown to permit tumor cells to escape apoptotic death (18). In tumorigenesis, many Hsp90 client proteins play a critical role in growth control, cell survival and tissue development, as they are able to facilitate an escape from normal proteolytic turnover through association with Hsp90-containing chaperone complexes, thereby overall promoting an oncogenic transformation (19-22).

Targeting Hsp90 for cancer therapy. Due to its central role in oncogenic signaling, Hsp90 provides an attractive target for the treatment of cancer. Inhibition of Hsp90 function results in simultaneous interruption of many signal transduction pathways, which are pivotal to tumor progression and survival. Recent results from experiments with the geldanamycin derivative 17-allylamino-17-demethoxygeldanamycin (17-AAG) have indicated that tumor cells utilize Hsp90 quite differently from normal cells, explaining the selectivity of the drug and suggesting a central role of Hsp90 in tumorigenesis (23). Much effort has been made in identifying novel and therapeutically applicable Hsp90 inhibitors (Table I) and the list is expanding rapidly. Several compounds have convincingly been shown to inhibit Hsp90 in *in vitro* and in *in vivo* systems, and 17-AAG as one of the earliest Hsp90 inhibitors has already been evaluated in phase I/II clinical trials (24-26). However, a new generation of non-geldanamycin small-molecule Hsp90 inhibitors is currently entering pre-clinical and clinical testing, hence additional clinical studies will be initiated in the near future, which underlines the importance for continued research on the molecular effects of Hsp90 targeted therapy (27-31). Only a profound knowledge of molecular biology will help to design effective inhibitors and successful clinical trials.

Effect of Hsp90 Inhibition of Gastrointestinal Tumor Cells

Several oncogenic signal transduction proteins are designated "clients" of Hsp90 and substantially rely on its function for maturation and/or stabilization. Notably, Hsp90 client proteins comprise members of the steroid receptor family and transcription factors, receptor tyrosine kinases, Src family members, serine-threonine kinases, cell cycle regulators, and many other proteins participating in oncogenic transduction pathways (Table II). The network of signal transduction pathways related to tumor progression and the involvement of Hsp90 client proteins is quite complex (Figure 1). Interestingly, Hsp90 clients comprise proteins that contribute to all of the six hallmarks of cancer as suggested by Hanahan and Weinberg, including self-sufficiency in growth signaling, insensitivity to antigrowth signals, evasion of apoptosis, sustained angiogenesis, tissue invasion and metastasis and a limitless replicative potential (32). Hence, Hsp90 appears to be a unique molecular target, as its inhibition ideally could simultaneously interfere with all of such key pathophysiological processes on which tumor cells depend for outgrowth and survival (23, 33).

Gastric cancer. Experimental and clinical studies have provided evidence that particularly the epidermal growth factor (EGF) acts as an important oncogenic growth factor in gastrointestinal carcinomas (34, 35). EGF, its receptor EGFR and also the human epidermal growth receptor-2 (HER-2/*neu*) are commonly overexpressed in human gastric carcinomas. Such expression has been associated with a poor prognosis of patients, suggesting that the EGFR system and HER-2/*neu* both play a critical role in the regulation of gastric cancer growth and angiogenesis (36, 37). Importantly, the induction of vascular endothelial growth factor-A (VEGF-A), known to be one of the most potent pro-angiogenic factors, can in cancer cells be mediated through activation of oncogenic signaling pathways, including phosphatidylinositol 3-kinase (PI3K)/Akt and mitogen-activated protein kinase/extracellular signal-regulated kinase (Erk). These signaling intermediates are down-stream of the EGF/EGFR system. In addition, both hypoxic and oncogenic activation of the transcription factor hypoxia-inducible factor-1α (HIF-1α) may induce VEGF-A, and thus promote tumor growth and metastasis (38, 39). The importance of targeting HIF-1α as a crucial regulator of gastric cancer growth and angiogenesis has previously been demonstrated (40). Nevertheless, the expression of Hsp90 *per se* has also been correlated with the development of gastric cancer and lymph node metastasis (41) and Hsp90 has therefore emerged as a promising target for gastric cancer therapy.

The function of the above mentioned oncogenic proteins, including mutated EGFR (42), HER-2 (43), mitogen-activated protein kinase/Erk, Akt (23), and the transcription factor HIF-1α (44), are highly dependent on the Hsp90 chaperoning function. In this context, our group recently demonstrated that blocking Hsp90 disrupts EGF-mediated signaling in gastric cancer cells *via* interference with the EGFR system (45). Both EGFR and HER-2 were down-regulated by this therapy, which consequently led to a substantial reduction in tumor growth and VEGF expression. Importantly, these experiments demonstrated profound antineoplastic efficacy of a low-dose anti-Hsp90 therapy, indicating that Hsp90 inhibitors probably do not require therapy at maximum tolerated doses (MTD) for achieving the desired biological effects *in vivo*. Similarly, potent growth inhibitory effects on gastric cancer cells have also been demonstrated with novel synthetic Hsp90 inhibitors (such as CNF2024), thus supporting the rationale for the use of Hsp90 targeting in gastric cancer (46).

Table I. *Overview of classes of Hsp90 inhibitors with representative substances and their site of action.*

Site of action	Class	Substance
NH_2-terminal ATPase	Benzoquinone	Geldanamycin
	Ansamycin	Herbimycin A
		Macbecin I, II
		17AAG
		17DMAG
	Macrolide	Radiciol
		Monocillin 1
		KF58333
	Purine scaffold	PU3
	Pyrazole	PU24FC1
		CCT018159
COOH-terminal ATPase	Coumarin antibiotic	Novobiocin
	Cross-linker	Cisplatin
Other	Histone	Depsipeptide
	deacetylase	Suberoylanilide
	inhibitor	Hydroxamic acid

17AAG: 17-allylamino-demethoxygeldanamycin; 17DMAG: 17-dimethylaminoethylamino-17-demethoxy-geldanamycin; KF58333: radicicol derivative.

Table II. *Overview of Hsp90 client proteins associated with oncogenesis.*

Kinases
Akt/PKB (protein kinase-B)
Bcr-Abl
Cdk4, Cdk6, Cdk9
Death domain kinase RIP
ErbB2 (and mutant EGF receptor)
IκB kinases α and β
c-MET (hepatocyte growth factor receptor)
IGF-IR (insulin-like growth factor-I receptor)
MEK (mitogen-activated protein kinase)
MOK, MAK, MRK
PDK1 (pyruvate dehydrogenase kinase 1)
Pim-1
Plk-1 (polo-like kinase-1)
FAK (focal adhesion kinase)
pp60v-src, c-src
src related tyrosine kinases: yes, fps, fes, fgr, lck
Raf-1, B-Raf
trkB
VEGFR2 (vascular endothelial growth factor receptor-2)
Wee 1, Swe 1
Transcription factors
Steroid receptors (GR, MR, ER, PR, AR)
HSF-1 (heat-shock factor-1)
p53
Stat3 (signal transducer and activator of transcription 3)
HIF-1α (hypoxia-inducible factor 1α)
NF-κB (nuclear factor κ-B)
Other
Apaf-1
Mdm2
Proteasome
Ral-binding protein 1
Survivin
SV40 large T-antigen
Telomerase

In addition to growth factor signaling, chronic inflammation appears to play a crucial role in the development and progression of several gastrointestinal carcinomas (47). In particular, malignant lesions developing in the stomach, such as mucosa-associated lymphoid tissue lymphoma (MALT lymphoma) and gastric adenocarcinoma, are closely associated with chronic persistent inflammation provoked by *Helicobacter pylori* infection (48). Among the cytokines induced in the gastric mucosa colonized by *H. pylori*, interleukin-8 (IL-8) is one of the major proinflammatory cytokines (49). Recently, it has been demonstrated that blockade of Hsp90 using geldanamycin completely inhibited *H. pylori*-induced IL-8 production due to deactivation of Erk and nuclear factor-κB (NF-κB), suggesting that blocking Hsp90 could abrogate *H. pylori*-mediated gastric carcinogenesis (50). Although this aspect will probably never be investigated, results from these experiments underline the importance of targeting Hsp90 in disrupting cytokine mediated signaling in gastric carcinogenesis.

In conclusion, the experience thus far in targeting Hsp90 in gastric cancer cells is encouraging, as it has been clearly demonstrated that gastric carcinomas harbor multiple molecular targets that are attractive for therapy with Hsp90 inhibitors. Hence, the addition of Hsp90 targeting compounds could lead to improved efficacy of current anti-neoplastic chemotherapy regimens.

Colorectal cancer. Colon cancer is of particular interest with respect to Hsp90 antagonists because of the high incidence of *KRAS* (oncogene) mutations, the constitutive activation of Ras/Raf/MEK/Erk signaling components and the proven sensitivity to specific inhibitors against these signal transduction pathways (51-53). The efficacy of Hsp90 inhibition has been shown in studies where 17-AAG effectively inhibited the growth of human colon cancer cells *in vitro* and delayed the growth of colon cancer xenografts *in vivo* (54). However, not only geldanamycin derivates, but also novel synthetic Hsp90 inhibitors have proven antitumoral efficacy against colon cancer growth, thereby emphasizing the value of targeting Hsp90 in colorectal carcinomas (55).

In particular, blocking Hsp90 in colon cancer cells leads to a reduction of Raf-1 expression and inhibition of both Erk1/2 and Akt phosphorylation, an effect that is also paralleled by an overall depletion of Akt protein levels (56). The signaling substrate Akt is essential in a number of survival pathways, including the PI-3K pathway and the signaling through NF-κB (57). Interestingly, K-*ras* and N-*ras* are both depleted by 17-AAG, a finding which may be predominantly important in colorectal cancer where K-*ras* gene mutations are frequently detectable and associated with poor outcome (51). Furthermore, it has been demonstrated, that both cytostatic and apoptotic events can be induced in human colon cancer cells, and certainly at the concentrations of Hsp90 inhibitors that are achieved in animal models and in the plasma samples of patients treated in phase I trials (56, 58).

Tissue invasion and metastasis to distant organ sites are major features of advanced colorectal cancer. However, the antimetastatic potential of Hsp90 inhibitors might be questionable, as some studies suggested a higher incidence of bone metastases when Hsp90 was being targeted in a model of breast cancer (59). Our group has therefore further addressed this issue and hypothesized that blocking oncogenic signaling with Hsp90 inhibitors would impair the metastatic behavior of colon cancer cells. In view of the fact that the EGFR and c-Met receptor (receptor to hepatocyte growth factor, HGF) systems both represent important mediators of colon cancer growth and metastasis (60-62), we focused on investigating the effects of Hsp90 inhibition on EGF- and HGF-induced signaling in human colon cancer cells and found that this approach substantially disrupted signaling. (63). Moreover, inhibition of Hsp90 also markedly reduced the HGF-mediated activation of the c-Met receptor and the phosphorylation of focal adhesion kinase (FAK), yet another essential mediator for cancer cell invasiveness. Importantly, blocking Hsp90 led to an up-regulation of the tumor suppressor and anti-metastatic factor activating transcription factor-3 (ATF3), however, the biological effects of this up-regulation remain to be elucidated. Nevertheless, in an *in vivo* model, the inhibition of Hsp90 significantly reduced tumor growth, vascularization, and the hepatic tumor burden in an experimental model of hepatic colon cancer growth (63).

Another aspect relates to the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL, also known as Apo2L), which has been identified as a potent inducer of cell death (64). Apoptosis induced by TRAIL is initiated through ligation and trimerisation of the cell functional death receptor (DR) TRAIL-R1 (DR4) and TRAIL-R2 (DR5) (65). Specificity for tumor cells makes TRAIL a promising candidate for cancer treatment alone or in combination, since the synergism with established chemotherapeutic drugs and radiation has been shown (66, 67). However, numerous cell types demonstrate resistance to TRAIL. The mechanisms of either sensitivity or resistance have not yet been fully elucidated; both Akt and NF-κB pathways may modulate cytotoxic responses. In regard to Hsp90 inhibition, the effects of TRAIL in combination with Hsp90 inhibition (17-AAG) have been analyzed in a series of colon cancer cell lines, and the activation of pathways to apoptosis have been characterized (68). Additivity, or synergism, of the TRAIL/17-AAG combinational therapy was demonstrated in all the cell lines. The sensitizing effect of 17-AAG was greater in the TRAIL-resistant cell lines, where the combination resulted in activation of both extrinsic and intrinsic apoptotic pathways, though with quantitative differences due to differential effects of 17-AAG on Akt and NF-κB (68). The results from these studies suggest that either Akt, or NF-κB may promote resistance to TRAIL in colon cancer cells and imply that blocking Hsp90 could prove suitable for overcoming TRAIL resistance in colon cancer (69).

A similar important aspect is that the antineoplastic efficacy of oxaliplatin, which is a substance now commonly used in multimodality chemotherapy regimens for treating advanced or metastatic (liver) colorectal cancer, might be improvable by the addition of Hsp90 inhibitors (63, 70, 71). Importantly, the resistance to oxaliplatin is in part mediated by mutation of p53, a common molecular event in colon cancer. The potential of Hsp90 inhibitors for improving the susceptibility to chemotherapy has initially been suggested by studies other than in colon cancer (72-75). Lately, Rakitina and co-workers showed that Hsp90 inhibitors promote oxaliplatin-dependent caspase activation and cytotoxicity by down-regulating antiapoptotic signaling through the transcription factor NF-κB in colon cancer cells *in vitro* (70). Moreover, recent data from the same group showed that Hsp90 inhibitors harbor the potential to inhibit G_1-S transition in p53-deficient colon cancer cells, thereby enhancing the cell cycle effects of oxaliplatin (71). Interestingly, the Hsp90 inhibitor associated enhancement of oxaliplatin cytotoxicity in colon cancer cell lines appears to be mediated through the inhibition of NF-κB activation (70). In a follow-up study, we further investigated the effects of 17-dimethylaminoethylamino-17-demethoxy-geldanamycin (17-DMAG) in combination with oxaliplatin on p53-wt and p53-deficient colon cancer cells, aiming to test whether these previous solely *in vitro* results translate into improved antineoplastic efficacy *in vivo*. Indeed, we were able to demonstrate that the Hsp90 inhibitor 17-DMAG improved the antineoplastic efficacy (growth inhibitory and proapoptotic effects) of oxaliplatin in p53-deficient colon cancer cells *in vivo*, suggesting Hsp90 antagonists to be valuable for improving the chemotherapy of advanced colorectal cancer, and potentially for the (neo-adjuvant) therapy of colorectal liver metastases (63).

Radiation therapy is an important component in the multimodal treatment regimen for advanced colorectal cancer. However, delivery of high-dose ionizing radiation (IR) is still limited due to the damage to surrounding normal tissues. One possible solution for this problem is to preferentially sensitize the tumor cells to IR, thus enabling dose reduction of IR in treatments. In this context, it has recently been reported that inhibition of a conventional mitogen-activated-protein-kinase (MAPK) pathway is an effective approach for sensitizing certain types of tumor cells to IR treatment, especially for those that are aggressively growing due to constitutively activated Erk1/2, *i.e.* HT-29 colon carcinoma cells. Most importantly, it has been demonstrated that concomitant treatment with the Hsp90 inhibitor 17-AAG leads to a radiosensitation effect in colon cancer cells (HT-29), even at a relatively low dose of irradiation (76). The potential of Hsp90 inhibitors to improve the efficacy of radiation therapy has also been proven in other preclinical models, including pancreatic cancer (77-79). Altogether, these different valuable aspects of targeting Hsp90 in colorectal cancer highlight the efficacy and relevance of this novel molecular approach, and could form the basis for future clinical investigation in this field.

Pancreatic cancer. Pancreatic cancer represents an aggressive cancer entity that remains largely resistant to conventional therapies, although the treatment of early stage pancreatic cancer with surgery and adjuvant chemotherapy may significantly improve survival. Therefore, the combination of molecular targeted therapies (*i.e.* trastuzumab, or cetuximab) with established chemotherapeutic agents (*i.e.* gemcitabine) is being actively investigated to improve survival (80). Against this background, pancreatic cancer has also been identified to be susceptible to Hsp90 inhibition, a promising novel form of targeted therapy. Studies concerning the biological behavior of pancreatic carcinoma have demonstrated that Hsp90 is overexpressed in pancreatic carcinoma, and pancreatic tumor cells were shown to contain high levels of *Hsp90* mRNA (17). Furthermore, Hsp90 has been found to be more highly expressed in poorly differentiated pancreatic adenocarcinoma and mucinous carcinoma, than in well to moderately differentiated ductal carcinoma (81). The observation that Hsp90 antagonists are able to potentiate chemotherapeutics has been confirmed in a pancreatic cancer cell line. However, during transition to serum-free conditions leading to nuclear localization of Hsp90, mutual inhibition rather than synergy with an Hsp90 antagonist and different cytotoxic agents has been seen. This could suggest that Hsp90 inhibition influences the nonspecific cell response towards a toxic stimulus (82). These results indicated that the benefit of potentiating chemotherapy may exclusively depend on the tumor environment and may even be reversed under certain conditions, such as a change in Hsp90 localization, which in *in vivo* tumors could contribute to the alleged specificity of Hsp90 inhibitors against tumor cells. As a functional consequence, prolonged combinational treatment may be ineffective, because a proportion of the cells will always be in the transition from cytoplasmatic to nuclear Hsp90, and thus be protected in the presence of the Hsp90 inhibitor. This aspect should be accounted for, when results from clinical trials with therapy using Hsp90 inhibitors in combination with other antineoplastic agents are being analyzed.

Since Hsp90 inhibitors are capable of interfering with multiple oncogenic signaling pathways, and insulin-like growth factor-I receptor (IGF-IR) and signal transducer and activator of transcription 3 (STAT3) signaling pathways are implicated in the progression of pancreatic cancer, our group recently hypothesized that blocking Hsp90 would impair IGF-I- and IL-6-mediated signaling, and thus could inhibit pancreatic cancer growth and angiogenesis (45). Indeed, inhibition of Hsp90 inhibited IGF-IR signaling by down-regulating IGF-IRβ and directly impairing IGF-IR phosphorylation. Moreover, hypoxia- and IL-6-mediated activation of HIF-1α, or STAT3, were substantially reduced, and a novel identified IL-6/STAT3/HIF-1α autocrine loop was effectively disrupted by inhibition of Hsp90 (45, 83). Furthermore, it has been validated, that Hsp90 blockade (17-DMAG) significantly reduced subcutaneous tumor growth and diminished STAT3 phosphorylation and IGF-IRβ expression in tumor tissues (45). Importantly, these potent effects were confirmed in an orthotopic pancreatic cancer model, and recent studies using inhibitors other than geldanamycin derivates support the hypothesis that Hsp90 represents a most promising target in pancreatic cancer (30, 31). In conclusion, the inhibition of Hsp90 could prove valuable for therapy of pancreatic cancer by eliciting anti-neoplastic efficacy and radio-sensitizing properties.

Effect of Hsp90 Inhibition on Non-malignant Cells

Hsp90 is also expressed by non-malignant cells that are involved in the process of tumor growth and metastasis. Recent findings from experiments with 17-AAG have demonstrated that tumor cells utilize Hsp90 quite differently from normal cells, providing an explanation for the selectivity of the drug. In one study, Kamal and coworkers have shown that Hsp90 derived from tumor cells elicits a 100-fold higher binding affinity to 17-AAG, than does Hsp90 in non-malignant cells (84). Tumor-associated Hsp90 is entirely present in the form of a multi-chaperone complex, thereby defining an activated high-affinity conformation that facilitates malignant progression. Importantly, solid malignancies not only consist of tumor cells, but also contain a great variety of other cell types, including endothelial cells, pericytes (vascular smooth muscle cells) and immune cells.

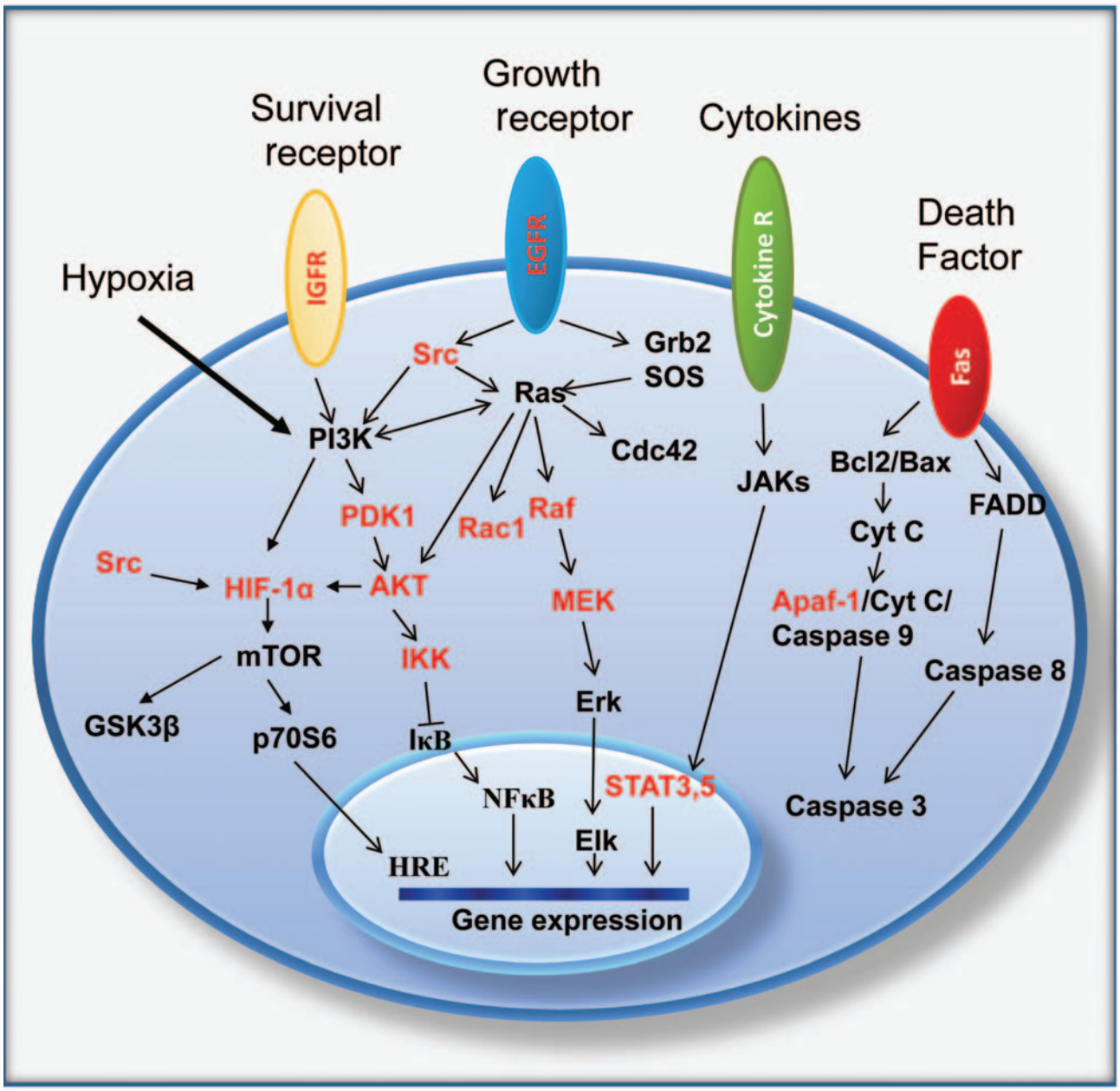

Figure 1. *Schematic illustration of signal transduction pathways related to tumor progression. Multiple key signaling components of oncogenic signaling pathways are designated Hsp90 clients (red), suggesting that targeting Hsp90 could represent a global and effective attack on oncogenic signaling cascades.*

This raises the question of how inhibition of Hsp90 would affect these types of cells.

Interestingly, Hsp90 inhibitors harbor the potential to affect pathways that are critically involved in the process of tumor angiogenesis, such as VEGFR and STAT3 (83, 85). Hence, anti-Hsp90 therapy could elicit antiangiogenic properties. Such antiangiogenic effect might occur indirectly in terms of modulating the expression of certain growth factors by tumor cells, or through direct effects on the endothelial cells (or pericytes). Recently, Kaur and colleagues addressed this important issue and found that the Hsp90 antagonist 17-DMAG inhibited fibroblast growth factor (FGF)-2 and VEGF-induced endothelial cell proliferation (85). Moreover, other endothelial cell functions related to the angiogenic process, such as migration, invasion into the extracellular matrix, and the ability to form capillary-like structures were

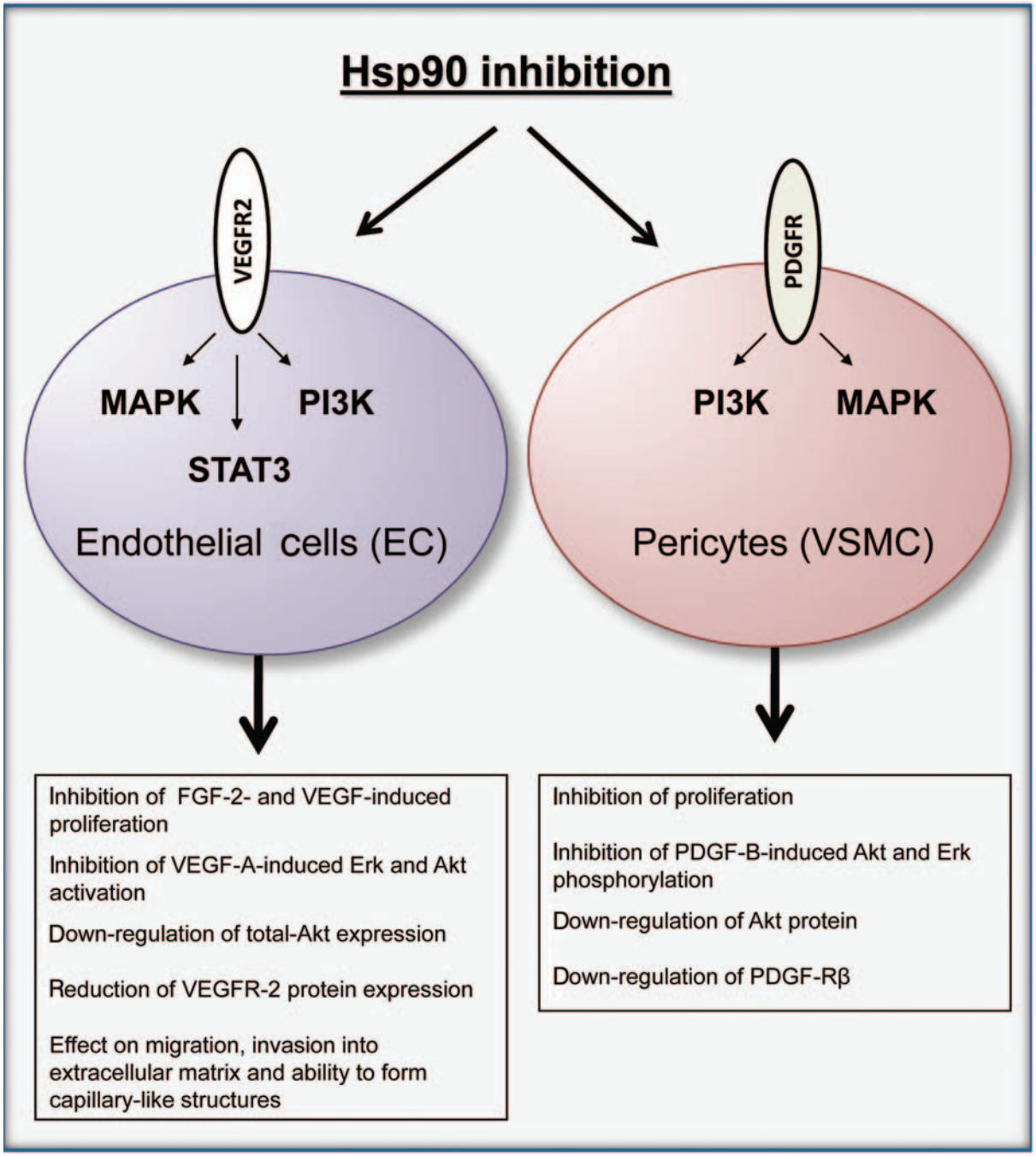

Figure 2. *Potential effects of Hsp90 inhibitors on non-malignant cells. Tumors consist of various cell types that overall define the tumor microenvironment, thereby modulating tumor growth and angiogenesis. Particularly interesting are the effects of Hsp90 targeting agents on non-malignant cells, such as endothelial cells (EC) and pericytes (vascular smooth muscle cells, VSMCs). Results from recent studies suggest that blocking Hsp90 also affects these cell types, thus eliciting antiangiogenic properties. However, higher therapeutic doses of Hsp90 inhibitors might be required for achieving effects on ECs and VSMCs (85, 86).*

also dramatically affected by 17-DMAG. In this context, blocking Hsp90 resulted in a degradation of Akt, c-Raf-1 and ERK protein kinases. These results confirm that targeting Hsp90 inhibits angiogenesis and the strong effects on endothelial cell functions indicate that the antiangiogenic activity of 17-DMAG could also be due to a direct effect on endothelial cells. Lang *et al*. additionally investigated whether Hsp90-inhibition would affect the proliferation and survival

Table III. *Clinical trials with Hsp90 targeting agents involving gastrointestinal malignancies (www.clinicaltrials.gov).*

Study	Conditions	Drug	Status
Safety study of IPI-504 in patients with gastrointestinal stromal tumors (GIST) or soft tissue sarcomas (STS)	Gastrointestinal stromal tumors (GIST), Soft tissue sarcomas (STS)	IPI-504	Recruiting
Gemcitabine and tanespimycin in treating patients with stage IV pancreatic cancer	Pancreatic cancer	Gemcitabine Tanespimycin	Not yet recruiting
PXD101 and 17-*N*-Allylamino-17-demethoxy-geldanamycin in treating patients with metastatic or unresectable solid tumors or lymphoma	Lymphoma, small intestine cancer, unspecified adult solid tumor	Belinostat Tanespimycin	Completed
17-*N*-Allylamino-17-demethoxygeldanamycin and bortezomid in treating patients with relapsed or refractory hematologic Cancer	Leukemia, lymphoma, small intestine cancer	Bortezomid Tanespimycin	Recruiting
17-*N*-Allylamino-17-demethoxygeldanamycin in treating patients with advanced epithelial cancer, malignant lymphoma, or sarcoma	Lymphoma, ovarian cancer, sarcoma, small intestine cancer, unspecified adult solid tumor	Tanespimycin	Completed
chemotherapy in treating patients with refractory advanced solid tumors or hematologic cancer	Bladder cancer, breast cancer, colorectal cancer, gastric cancer, head and neck cancer, kidney cancer, leukemia, lung cancer, melanoma, ovarian cancer, prostate cancer, unspecified adult solid tumor	Tanespimycin	Completed
17- Dimethylamino-17-demethoxygeldanamycin (17-DMAG) in treating patients with an advanced solid tumor or lymphoma	Lymphoma, small intestine cancer, unspecific adult solid tumor	17-DMAG	Active, not recruiting
17-DMAG in treating patients with metastatic or unresectable solid tumors	Breast cancer, colorectal cancer, gastric cancer, head and neck cancer, kidney cancer, melanoma, ovarian cancer, prostate cancer, unspecified adult solid tumor	17-DMAG	Active, not recruiting

of gastric fibroblasts and vascular smooth muscle cells, demonstrating that Hsp90 inhibitors indeed have a direct effect on these non-malignant cell types, although higher doses of Hsp90 inhibitors might be required for targeting pericytes (with a direct cytotoxic effect) for antiangiogenic therapy *in vivo* (45, 86). Figure 2 summarizes the effects of Hsp90 inhibition in endothelial cells and pericytes.

Regarding aspects of the immune system, it recently has been demonstrated that Hsp90 inhibitors may act as immuno-suppressants by blocking T-cell signaling through counteracting both the T-cell receptor- (87, 88) and CD28-mediated (89) signal transduction. Additionally, Hsp90 inhibition also seems to inhibit B-cell responses (90). This could imply that blocking Hsp90 could compromise the immune response to tumors. On the other hand, results from experiments by Sreedhar *et al*. suggest that Hsp90 inhibition seems to be an effective method of cell sensitization to both hypoxia- and immune-mediated cell lysis, a phenomenon that may help the immune system to attack tumor cells and which illustrates a new character to the mechanism of action of Hsp90 inhibitor drug candidates (91). However, at this point it is not absolutely clear what role these alterations of the immune response exactly play in the context of targeting Hsp90 for cancer therapy.

Clinical Trials

The potential value of Hsp90 as a molecular target in cancer therapy is reflected by the continued initiation of novel clinical trials. For example, studies with 17-AAG and the more potent, water-soluble geldanamycin analogue 17-DMAG, are ongoing, or have been completed already. These studies may serve as a "proof of principle" for the hypothesis that Hsp90 function can be modulated pharmacologically in humans (24, 25, 92, 93). Moreover, several phase II trials of malignancies in which Hsp90 clients are known to play an important role (*i.e*. melanoma, breast cancer, renal cell carcinoma) are now active (an updated list of recruiting, active or completed phase I/II trials with Hsp90 inhibitors can be found on the website www.clinicaltrials.gov). Another field of interest involves the feasibility and efficacy of combining Hsp90 inhibition with conventional chemotherapy (*i.e*. gemcitabine, docetaxel, irinotecan), other molecular targeted drugs (*i.e*. bortezomid, imatinib, rituximab), or radiation therapy. Currently, several phase Ib trials are active to investigate such specific drug combinations, as for example the combination of Hsp90 inhibition with bortezomid in patients with relapsed-refractory multiple myeloma or combination with docetaxel in patients

with advanced solid tumors. A summary of current clinical studies under way to examine Hsp90 inhibition in gastrointestinal malignancies as single or combination treatment is provided in Table III.

Furthermore, molecular target effects (*i.e.* increases in Hsp70) have been demonstrated (23), and preclinical studies suggested feasible biological monitoring in terms of signaling pathway analysis in tumor samples from needle-biopsy (46). Most importantly, disease stabilization as a consequence of Hsp90-targeted therapy has also already been observed (94). Ongoing studies of Hsp90 inhibition in an alternate schedule and in combination with chemotherapeutic agents will more accurately define effects of Hsp90 blockade on client proteins and chemosensitivity.

In summary, this article demonstrates the crucial role of Hsp90 as an anticancer drug target because of its central importance in maintaining the function of key oncogenic client proteins. With regard to gastric, colorectal and pancreatic cancer, Hsp90 inhibitors have shown promising antitumor activity in *in vitro* and in *in vivo* preclinical modes. Furthermore, it has been pointed out that Hsp90 inhibitors not only elicit potent antineoplastic effects and antiangiogenic properties, but also render tumor cells susceptible to chemotherapy and/or radiation therapy. This defines a valuable advantage of Hsp90-targeting agents to be used in combination with multimodality therapies. Currently, several phase I and II trials are investigating the anticancer efficiency of Hsp90 inhibitors, as a single or combination therapy, and results from these studies will provide important information on tumor biology. In particular, the identification of adequate biomarkers will be most important for successful design and monitoring of Hsp90-targeted therapies.

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