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Role of the hypoxic tumor microenvironment in the resistance to anti-angiogenic therapies

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Abstract

Angiogenesis, a key process for the growth of human cancers, has recently been exploited for the development of a novel class of cancer therapeutics that was thought to have wide applications and *not* to induce resistance in the clinical setting. Indeed, anti-angiogenic therapy has become an important option for the management of several human malignancies. However, a significant number of patients either do not respond to anti-angiogenic agents or fairly rapidly develop resistance. In addition, the benefit of anti-angiogenic therapy is relatively short-lived and the majority of patients eventually relapses and progresses. Several mechanisms of resistance to anti-angiogenic therapy have been recently proposed. The current review focuses on the role of intratumor hypoxia as a mechanism of resistance to anti-angiogenic agents and speculates on therapeutic approaches that might circumvent resistance and thereby improve clinical outcome.

Keywords

Angiogenesis; VEGF; hypoxia; HIF-1; bevacizumab; cancer therapeutics; topotecan; mTOR inhibitors

1. Introduction

Studies over the past 30 years have shown that angiogenesis is an important process contributing to the progression of cancer from an *in situ* lesion to invasive and metastatic disease, providing the rationale for the development of anti-angiogenic therapies (Kerbel et al., 2002; Folkman, 2007). To this date, several anti-angiogenic approaches have been investigated in animal models as well as in the clinic. Targeting the vascular endothelial growth factor (VEGF)/VEGF receptor pathway, alone or in combination with chemotherapy, has shown clinical benefit in patients with metastatic colorectal cancer, advanced non-small cell lung cancer, renal cell carcinoma, hepatocellular carcinoma and metastatic breast cancer (Ferrara, 2005; Shojaei et al., 2007a; Ellis et al., 2008b). Antiangiogenic agents are then an integral component of current therapeutic approaches of combination chemotherapy and/or molecularly targeted therapies.

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Although anti-angiogenic therapy is becoming an important option for the treatment of cancer, its systematic application remains problematic because of both poor understanding of its mechanisms of action and occurrence of resistance (Jain et al., 2006). Indeed, a significant fraction of patients does not respond to anti-angiogenic therapy (Burris et al., 2008), whereas those who respond have a relatively modest survival benefit. In addition, despite disease stabilization and an increase in the proportion of progression free patients, tumors eventually become resistant to anti-angiogenic agents and relapse (Bergers et al., 2008; Ellis et al., 2008a; Kerbel, 2008; Shojaei et al., 2008b). In the end, which patients may potentially benefit from the addition of an anti-angiogenic agent to the therapeutic regimen remains poorly understood.

Multiple mechanisms may account for the activity of anti-VEGF agents in cancer patients including, but not limited to, their effect on tumor vasculature (Ellis et al., 2008b). Evidence has been provided supporting both a vascular regression, which is presumably associated with increased intratumor hypoxia (Kerbel et al., 2002) and a so-called “normalization” of tumor vasculature, with a consequent decrease in interstitial pressure and better delivery of chemotherapy (Jain, 2005). These conflicting and still largely controversial observations emphasize how important it is to better understand the effects of anti-angiogenic agents on the tumor microenvironment to eventually further characterize the mechanisms that mediate resistance.

Hypoxia, areas of low oxygen levels, is a hallmark of solid tumors due to an imbalance between oxygen delivery and consumption (Brown et al., 2004). The presence of hypoxia in solid tumors is associated with resistance to radiation therapy and chemotherapy, selection of more invasive and metastatic clones and poor patient prognosis (Harris, 2002; Hockel et al., 2001). Hypoxia Inducible Factor-1 (HIF-1) is a master regulator of cellular adaptation to oxygen deprivation and may act as a survival factor of hypoxic cancer cells, primarily by activating transcription of genes involved in angiogenesis, glycolytic metabolism, oxygen consumption, migration and invasion (Semenza, 2007). HIF-1 is a heterodimeric protein consisting of a constitutively expressed HIF- β subunit and a HIF- α subunit, the expression of which is regulated by the cellular O₂ concentration (Wang et al., 1995). Under normoxic conditions, HIF-1 α is continuously hydroxylated by oxygen-dependent prolyl hydroxylases, and targeted for ubiquitination and proteasomal degradation (Pouyssegur et al., 2006). On the contrary, under hypoxic conditions the HIF- α subunit is stabilized and translocates to the nucleus where it dimerizes with HIF-1 β (also known as aryl hydrocarbon receptor nuclear translocator, ARNT) and, by binding to hypoxia responsive elements (HRE), activates transcription. Expression of HIF-1 α has been demonstrated in many human cancers and is associated with poor prognosis and treatment failure (Koukourakis et al., 2006; Aebersold et al., 2001; Birner et al., 2000; Birner et al., 2001; Bos et al., 2003).

In this review we will discuss the main mechanisms that have been implicated in resistance to antiangiogenic agents with particular emphasis on the role that intratumor hypoxia and activation of HIF-1 dependent responses might play. Our conclusions may contribute not only to a better appreciation of the role of the tumor microenvironment in mediating resistance to antiangiogenic agents but also to the design of novel therapeutic approaches.

2. Mechanisms of resistance to anti-angiogenic therapy

Resistance to antiangiogenic agents is a complex phenomenon that can be broadly classified as intrinsic and acquired resistance.

2.1. Intrinsic resistance

A substantial fraction of patients treated with anti-angiogenic agents, including bevacizumab, sorafenib or sunitinib, failed to show even a transient clinical benefit (Burris et al., 2008; Batchelor et al., 2007). This lack of clinical benefit could be interpreted as a rapid adaptation to and escape from the effects of anti-angiogenic agents or, in some cases, the result of pre-existing resistance. It is conceivable that tumors, during their development, activate pathways that makes them intrinsically resistant to anti-angiogenic therapy, such as redundancy of angiogenic factors (FGFs, PDGFs, PIGF) (Relf et al., 1997; Fischer et al., 2007), increased metastatic and invasive potential without angiogenic switch (Casanovas et al., 2005), high levels of infiltrating inflammatory cells, which produce a number of pro-angiogenic factors (Shojaei et al., 2008b) or hypovascularity, such as in pancreatic ductal adenocarcinoma (Saif, 2007).

2.2. Acquired resistance

Considering the results of both pre-clinical and clinical research and the overall modest effect of anti-angiogenic therapy in patients with solid tumors, it is now widely recognized that tumors rapidly adapt to the effects of anti-VEGF agents to resume growth. Apart from few instances of intrinsic resistance, most tumors acquire resistance to anti-angiogenic therapies by up-regulating pathways that sustain tumor growth and progression. Acquired resistance to anti-angiogenic agents has been attributed to a number of potential mechanisms, including

- i. up-regulation of alternative pro-angiogenic signals,
- ii. increased production of pro-angiogenic factors by stromal cells,
- iii. recruitment of bone marrow derived pro-angiogenic cells,
- iv. increased vascular pericyte coverage,
- v. activation of an invasive phenotype.

Interestingly, hypoxia and HIF-dependent responses most likely play an essential role in several of these adaptive mechanisms. For instance, elevated CA9 (carbonic anhydrase 9, a HIF-1 target gene) and HIF-2 α levels are inversely correlated with response to bevacizumab and irinotecan in malignant astrocytoma (Sathornsumetee et al., 2008), consistent with the possibility that intra-tumor hypoxia may be an important factor in mediating resistance to anti-angiogenic agents.

2.2.1. Upregulation of compensatory pro-angiogenic pathways—A compensatory increase of fibroblast growth factors (FGFs) was one of the first mechanisms of resistance identified in preclinical models. In the Rip1-Tag2 mouse model of pancreatic neuroendocrine cancer, researchers showed that an antibody that blocks VEGFR2 signaling (DC101) induced tumor stasis and a decrease in vascular density. However, this response was transitory and was followed by a phase of tumor re-growth, during which tumor vasculature was increased, indicating active tumor angiogenesis (Casanovas et al., 2005). Interestingly, relapsing tumors expressed high levels of FGF2 and SDF-1, genes that are controlled by HIF-1 (Calvani et al., 2006; Hirota et al., 2006). The potential relevance of these findings is supported by clinical data that reported the induction of FGF2 in serum of patients that progressed on anti-VEGF therapy (Batchelor et al., 2007).

In both preclinical and clinical studies, placental growth factor (PIGF) was shown to be up-regulated following anti-VEGF therapy (Batchelor et al., 2007). Blockade of PIGF with a monoclonal antibody reduced tumor angiogenesis and metastasis in mouse models, inhibited the recruitment of tumor infiltrating macrophages and did not increase tumor hypoxia, in tumors sensitive and resistant to anti-VEGF therapy (Fischer et al., 2007). In this setting, anti-

PIGF therapies might play a complementary role with anti-VEGF therapies. However, clinical development of VEGF-Trap (that binds both VEGF and PIGF) did not show, thus far, any additional benefit compared to bevacizumab (a humanized antibody that binds VEGF-A).

Recent data emphasize the role of the plasma-membrane bound Notch ligand/receptor system in the development of resistance to anti-angiogenic therapy. The activation of this signaling pathway leads to a more mature vasculature. Inhibition of Notch/Delta-like ligand 4 (Dll4) signaling induces an increase in vessel density, but a paradoxical decrease in tumor perfusion and increase in intra-tumor hypoxia, due to a decrease in vessel function (Thurston et al., 2007; Ridgway et al., 2006; Sainson and Harris, 2008). Moreover, tumors that have an intrinsic resistance to anti-VEGF agents appear to be sensitive to inhibition of Dll4 (Yan et al., 2007). Dll4 has been shown to be under control of HIF-1 (Diez et al., 2007), emphasizing again the role that the hypoxic pathway may play in mediating resistance to anti-angiogenic therapy.

2.2.2. Production of pro-angiogenic factors by stromal cells—Reduced efficacy of anti-angiogenic therapy may be due to the involvement of the stromal compartment in tumor angiogenesis. The stroma encompasses a variety of cellular types, including fibroblasts, pericytes, mesenchymal and hematopoietic cells, that support tumor growth by several mechanisms, including direct contribution to the tumor vasculature and release of VEGF and MMP9 (Shojaei et al., 2008b). In particular, tumor associated fibroblasts (TAFs) are thought to play a major role in tumor growth and possibly in resistance to anti-angiogenic therapy. Studies in xenograft models have demonstrated that blocking only human VEGF was not sufficient to inhibit tumor growth due to the production of murine VEGF by the stroma (including TAFs) (Liang et al., 2006). In addition, recent evidence has been provided that TAFs from tumors resistant to anti-VEGF therapy can support tumor growth and angiogenesis by producing PDGF-C, proposing yet another potential mechanism of resistance to anti-angiogenic therapy (Crawford et al., 2009). Contrary to findings described above, conditional deletion of VEGF from myeloid infiltrating cells did actually increase tumor growth in mouse models, raising questions about the fundamental contribution of infiltrating myeloid cells to tumor angiogenesis (Stockmann et al., 2008).

Taken together, these observations emphasize the role that the tumor microenvironment plays in drug resistance in general and to anti-angiogenic agents in particular, strongly suggesting that the stromal cellular component needs to be taken into account in order to improve efficacy of anticancer therapies.

2.2.3. Recruitment of bone marrow derived pro-angiogenic cells—Induction of intratumor hypoxia during therapy with antiangiogenic agents may lead not only to an increase in the production of pro-angiogenic factors by tumor and stromal cells, but also to recruitment of bone marrow derived cells (BMDCs) that have the capacity to elicit angiogenesis and tumor growth. Pro-angiogenic BMDCs consist of vascular progenitors (such as endothelial and pericytes progenitors) and vascular modulators (such as tumor associated macrophages, immature monocytic cells, myeloid cells) (Kerbel, 2008). Evidence that hypoxia induced the recruitment of BMDCs were first obtained in an experimental model of ischemia, in which endothelial progenitor cells and other BMDCs expressing CXCR4 were recruited to the ischemic tissue by a HIF-1 α -dependent increase of downstream effectors SDF-1 and VEGF (Ceradini et al., 2004). Moreover, a marked mobilization of circulating bone marrow derived cells occurs rapidly after treatment of tumor bearing mice with vascular disrupting agents, along with massive induction of tumor hypoxia (Shaked et al., 2006). In this setting, circulating endothelial cells have been shown to contribute to the rapid regrowth of tumors (Shaked et al., 2006). In addition, there is evidence that this mechanism may operate in glioblastoma patients undergoing anti-angiogenic therapy. Indeed, increase of FGF2, SDF-1 and viable circulating endothelial cells (CECs) was observed when tumors progressed following AZD2171 treatment

(Batchelor et al., 2007) and CECs increased after sunitinib treatment of renal cell cancer patients (Vroling et al., 2009).

More recently, it has been suggested that a specific myeloid cell population migrates to tumors and mediates tumor angiogenesis and resistance to anti-VEGF agents (Shojaei et al., 2007b). Interestingly, production of G-CSF, IL6 and SDF-1 by tumor and stromal cells mediated the mobilization of CD11b⁺Gr1⁺ myeloid cells to the tumor, where they elicited angiogenesis conferring resistance to anti-VEGF therapy (Shojaei et al., 2007b; Shojaei et al., 2008a). Moreover, CD11b⁺ myeloid cells have been shown to be recruited at premetastatic sites in response to SDF-1 and lysyl oxidase (LOX) gradients and to promote tumor metastasis through the production of MMP2 (Yang et al., 2008; Erler et al., 2009).

2.2.4. Increased pericytes coverage of the vasculature—Pericytes are involved in vascular stability and provide survival signals to endothelial cells. Inhibition of VEGF signaling results in survival of endothelial cells that are in strict contact with pericytes in “mature vessels” (Benjamin et al., 1999). Anti-VEGF therapy may lead to endothelial cell apoptosis and pruning of immature tumor vasculature (without pericytes coverage), but also to an increase in angiopoietin 1 that enhances pericyte recruitment to the vessels, therefore reversing the effect of anti-VEGF therapy (Winkler et al., 2004). Interestingly, HIF-1 regulates the expression of PDGF, PAI-1, angiopoietin 1 and Tie-2, genes involved in the recruitment of pericytes and their interaction with endothelial cells (Hirota et al., 2006). Based on the role of pericytes on vessels stabilization and endothelial cell survival, it has been proposed to combine anti-VEGF agents with agents that prevent the recruitment of pericytes to the tumor vasculature (such as inhibitors of PDGF signaling). Indeed, a number of studies has shown that targeting both pericytes and endothelial cells (PDGF-R and VEGFR inhibitors) may lead to synergistic inhibition of tumor growth (Bergers et al., 2003). Conversely, recent evidence suggests that targeting pericytes in the tumor vasculature may lead to disruption of vessel integrity, enabling tumor cells to transit into the circulation system and metastasize (Xian et al., 2006). Moreover, a negative rather than positive effect of VEGF on pericyte function and vessel maturation has also been recently suggested, adding complexity to the potential effects of VEGF/PDGF modulation (Greenberg et al., 2008). Due to the similarities between VEGFRs and PDGFRs, many receptor tyrosine kinase inhibitors (sunitinib, sorafenib) that target VEGFRs also inhibit PDGFR functions. The clinical benefit of targeting both endothelial cells and pericytes remains to be determined.

3. The hypoxic tumor microenvironment and response to anti-angiogenic agents

The fine balance between oxygen and nutrients supply by blood vessels and proliferation of cancer cells determines the onset of intra-tumor hypoxia and the induction of the angiogenic switch. Tumors that fail to activate angiogenic pathways may remain dormant and do not progress. The key regulator of hypoxia-induced angiogenesis is the transcription factor hypoxia inducible factor (HIF)-1. Multiple HIF-1 target genes are involved in different steps of angiogenesis: induction of growth factors and their receptors (VEGF, PlGF, Flt-1), increased vascular permeability (VEGF, Flt-1, angiopoietin 2, Tie-2), extracellular matrix remodeling (MMPs, collagen prolyl-4-hydroxylase, uPAR), migration and proliferation of endothelial cells (VEGF, PlGF, FGF2, angiopoietin 1, MCP-1, PDGF, SDF-1, CXCR4), endothelial cell sprouting (angiopoietin 2, Tie-2), endothelial tube formation and cell-to-cell interaction (VEGF, PlGF, angiopoietin 1, integrins) and recruitment of and interaction with pericytes (PDGF, PAI-1, angiopoietin 1, Tie-2) (Hirota et al., 2006).

Considering the key role of intra-tumor hypoxia in the angiogenic switch, it is conceivable that hypoxic tumors, expressing elevated levels of VEGF, might respond to anti-angiogenic

therapies, while well oxygenated tumors might not. Several clinical trials sought to assess the relevance of VEGF expression as a prognostic marker to anti-VEGF therapy but, overall, elevated VEGF blood levels prior to therapy were not predictive of response to anti-angiogenic agents (Sessa et al., 2008), which may be partly due to relative insensitivity of blood VEGF concentration to the VEGF secretion rate of tumors (Stefanini et al., 2008). It is important to point out that anti-angiogenic agents have been used in patients with advanced metastatic disease, in which the angiogenic switch has already occurred. Application of these agents in early stages of disease might be more effective.

Although the mechanism of action of anti-VEGF agents is complex and involves several aspects of endothelial, immune and cancer cells biology, one of the initial responses to anti-angiogenic therapies is the decreases in vessels number and function and a consequent increase in intra-tumor hypoxia (Ellis et al., 2008b). Indeed, animal models have provided evidence that induction of HIF-1 dependent genes may discriminate between tumors that respond or do not respond to anti-VEGF therapy (Dang et al., 2008). In addition, treatment with anti-angiogenic agents increases plasma levels of VEGF and PlGF in patients, an increase that has been proposed as a predictive biomarker for tumor response (Bocci et al., 2004; Bertolini et al., 2007; Pathak et al., 2008). If, on the one hand, activation of hypoxia-inducible pathways has been associated with administration of anti-VEGF agents, on the other hand, it may contribute to resistance and tumor progression.

4. Targeting the hypoxic tumor microenvironment to overcome resistance to anti-angiogenic therapy

Based on the evidence discussed so far, it is conceivable that the increase in intra-tumor hypoxia induced by antiangiogenic agents may be part of a fundamental mechanism by which cancer cells adapt to the decreased blood supply and escape from its potential detrimental effects. The biological consequences of intra-tumor hypoxia and its potential role for the development of tumor-specific therapeutics have been subject of investigation for many years. However, only over the last two decades the molecular mechanisms underlying intra-tumor hypoxia have been, at least in part, elucidated. As mentioned above, HIF-1 is a critical transcription factor for the response of mammalian cells to oxygen deprivation and is a likely candidate for the induction of compensatory pathways activated by cancer cells under hypoxic conditions. Indeed, HIF-1 mediates the transcription of genes involved in angiogenesis, glycolytic metabolism, oxygen consumption, migration and invasion (Semenza, 2007). Considering that HIF-1 α expression in human cancers has been associated with poor prognosis and treatment failure, efforts have been made to identify small molecule inhibitors of HIF-1 (Melillo, 2007; Powis et al., 2004) and evidence has been provided that inhibition of HIF-1 in xenograft models is associated with decreased angiogenesis and delayed tumor growth (Rapisarda et al., 2004b). While expression of HIF-1 α in the majority of solid tumors is focal and heterogeneous, detected predominantly in perinecrotic areas, its expression may be potentially augmented by administration of antiangiogenic agents and concomitant increase in intratumor hypoxia. Higher levels of HIF-1 in the tumor microenvironment may not only contribute to drug resistance in general, for instance by increasing *MDR-1* expression or affecting sensitivity to radiation therapy and DNA damaging agents (Brown et al., 2006; Brown et al., 2006; Moeller et al., 2004; Moeller et al., 2005), but also to resistance to anti-angiogenic agents in particular.

4.1. Activation of survival pathways

It is intuitive that consistently higher levels of HIF-1 in the tumor microenvironment may activate survival pathways in cancer cells, in which the apoptotic program is genetically altered. HIF-1 induces glycolytic enzymes, responsible for a switch to glycolysis even in the presence of oxygen (Warburg effect), and PDK-1, which inhibits pyruvate dehydrogenase and affects

mitochondrial biogenesis, overall conferring a survival advantage to oxygen and nutrient-deprived cancer cells (Kim et al., 2006). In addition, as discussed above, HIF-1 induces a number of angiogenic factors that may contribute to survival of endothelial cells and bypass the inhibition of the pathway targeted by the antiangiogenic strategy (Figure 1).

4.2. Activation of an invasive phenotype

Another possible mechanism of tumor cell resistance to anti-angiogenic agents is the acquisition of a more invasive phenotype. HIF-1 induces genes that control invasion and metastasis, including but not limited to c-met (Pennacchietti et al., 2003), CXCR4 (Staller et al., 2003; Schioppa et al., 2003) and LOX (Erler et al., 2006), inhibits the expression of E-cadherin and contributes to epithelial mesenchymal transition (EMT) (Krishnamachary et al., 2006; Sabbah et al., 2008). Indeed, expression of HIF-1 α in human tumors correlates with metastasis (Rankin et al., 2008). Interestingly, recent studies implicate the acquisition of an invasive phenotype in glioblastoma patients who have developed multifocal tumor recurrence during the course of anti-angiogenic therapy (Norden et al., 2008; Narayana et al., 2009), consistent with preclinical data suggesting that reduction of tumor vasculature and increase in intra-tumor hypoxia might result in enhanced tumor cells invasiveness. In particular, recent evidence in preclinical models suggests that anti-VEGF therapies, while inhibiting the growth of primary tumors, might promote tumor invasiveness and metastasis and be associated with shorter survival (Ebos et al., 2009; Paez-Ribes et al., 2009; Loges et al., 2009).

4.3. Therapeutic implications

Given that HIF-1-dependent genes may play key roles in multiple mechanisms implicated in the resistance to anti-VEGF therapies, combination of these agents with HIF-1 inhibitors might result in inhibition of adaptive pathways and increased therapeutic efficacy. Likewise, activity of HIF-1 inhibitors might be maximized in the presence of therapy-induced intratumor hypoxia.

HIF-1 is an attractive, yet challenging, target for the development of pharmacological inhibitors (Melillo, 2007). Several HIF-1 inhibitors identified so far are either FDA approved or are in early clinical development and could be potentially used for combination studies with anti-angiogenic agents (Table 1). Proposed mechanisms of action of these HIF-1 inhibitors are shown in Figure 1.

HIF-1 α accumulation is controlled primarily at the level of protein degradation or protein translation. HIF-1 α is a client protein of Hsp90 and Hsp90 inhibitors (17-AAG, 17-DMAG) have been shown to increase HIF-1 α protein degradation (Isaacs et al., 2002; Majeesh et al., 2002; Li et al., 2009). On the other hand, several agents may affect HIF-1 α translation, including the topoisomerase I inhibitors topotecan (Rapisarda et al., 2004a) and EZN-2208, a pegylated form of SN38 (Sapra et al., 2008), the small molecule PX-478 (Welsh et al., 2004) and the mTOR inhibitors temsirolimus (CCI-779) and everolimus (RAD001) (Del Bufalo et al., 2006; Wan et al., 2006). Additional mechanisms of action of HIF-1 α inhibition that have been proposed include modulation of HIF-1 α mRNA levels using antisense oligonucleotides (EZN-2968) (Greenberger et al., 2008) or the AhR ligand aminoflavone (Terzuoli E. and Melillo G., *submitted for publication*) and inhibition of HIF-1 DNA binding by echinomycin (Kong et al., 2005) and doxorubicin (Lee et al., 2009).

We have described a number of small molecule inhibitors of HIF-1 that act by distinct mechanisms (Trisciuglio et al., 2008; Creighton-Gutteridge et al., 2007; Park et al., 2006; Kong et al., 2005; Rapisarda et al., 2004a; Rapisarda et al., 2002) and demonstrated inhibition of HIF-1 expression and activity in xenograft models (Rapisarda et al., 2004b). To test the hypothesis that anti-VEGF therapies may be more efficacious in combination with HIF-1 inhibition, we combined topotecan, a topoisomerase I inhibitor that down-regulates HIF-1 α

protein *in vitro* and *in vivo* (Rapisarda et al., 2004a; Rapisarda et al., 2004b), with bevacizumab in a glioma xenograft model. Indeed, we found that inhibition of HIF-1 α by topotecan in a hypoxic stressed tumor microenvironment resulted in a more pronounced anti-tumor effect, relative to either agent alone. The effects on tumor growth were associated with significantly decreased HIF-1 transcriptional activity and reduced tumor cell proliferation (Rapisarda et al., *manuscript submitted*), consistent with the hypothesis that targeting HIF-1 α activity may abrogate compensatory pathways required for cancer cell survival. In this regard, it is interesting to point out that the combination of bevacizumab and irinotecan (another topoisomerase I inhibitor that also inhibits HIF-1) has shown clinical benefit in glioblastoma patients with a 6-month overall survival of 62–77% (Vredenburgh et al., 2007; Chen et al., 2007). Interestingly, another recent study showed cooperativity of irinotecan with the mTOR inhibitor rapamycin in an *in vivo* and *in vitro* colon cancer cell model through inhibition of HIF-1 α accumulation (Pencreach et al., 2009).

5. Conclusion and perspectives

The excitement for novel therapeutic strategies approaching the clinical arena is invariably tempered by the complexity of translating findings from preclinical models to cancer patients. Clinical trials with anti-angiogenic agents have initially generated great enthusiasm for the potential universal application of this therapeutic approach to human cancers. However, the premise that the efficacy of anti-angiogenic agents would not be limited by the inevitable occurrence of drug resistance has turned out to be a hopeful but incorrect prediction. Evidence discussed in this review emphasizes the role of the tumor microenvironment in determining resistance to anti-angiogenic agents and points to the need for a better understanding of changes effected by these targeted therapies as a way to optimize combination strategies. The rapid translation from preclinical models to the clinical setting of promising and validated approaches targeting the tumor microenvironment may be a way to expedite the development of more effective antiangiogenic combination therapies.

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Figure 1. Schematic representation of the potential interaction between the HIF-1 pathway and anti-angiogenic agents. HIF-1 inhibitors, with potential mechanisms of action, are indicated.

Table 1

HIF-1 inhibitors in clinical development with potential mechanisms of action.

Agent	Target	Mechanism	Reference
EZN-2968	mRNA	HIF-1 α mRNA degradation	Greenberger, 2008
Aminoflavone	AhR	HIF-1 α mRNA processing	Terzuoli, <i>submitted</i>
Topotecan	Topoisomerase 1	Translation	Rapisarda, 2004
EZN-2208	Topoisomerase 1	Translation	Sapra, 2008
PX-478	unknown	Translation	Welsh, 2004
Temsirolimus	mTOR	Translation	Del Bufalo, 2006
Everolimus			Wan, 2006
17AAG	HSP90	Degradation	Isaacs, 2002
17DMAG			Mabjeesh, 2002
Echinomycin	DNA	DNA binding	Kong, 2005
Doxorubicin	DNA	DNA binding	Lee, 2009