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**Molecular Pathways**

**Tumor and Host-Mediated Pathways of Resistance and Disease Progression in Response to Antiangiogenic Therapy**

John M. L. Ebos,1,2 Christina R. Lee,1 and Robert S. Kerbel1,2

**Abstract**

Despite early benefits seen in cancer patients treated with antivascular endothelial growth factor (VEGF) pathway-targeted drugs, the clinical benefits obtained in terms of progression-free or overall survival have been more modest than expected. This outcome is, at least in part, due to antiangiogenic drug resistance mechanisms that involve pathways mediated largely by the tumor, whether intrinsic or acquired in response to therapy, or by the host, which is either responding directly to therapy or indirectly to tumoral cues. The focus of this review is to distinguish, where possible, between such host and tumor-mediated pathways of resistance and discuss key challenges facing the preclinical and clinical development of antiangiogenic agents, including potential differences in drug efficacies when treating primary tumors or various stages of metastatic disease. (Clin Cancer Res 2009;15(16):5020-5)

**Background**

The concept of targeting the tumor's vasculature to block its growth has been validated clinically as an anticancer strategy with the approval of three targeted drugs that disrupt the vascular endothelial growth factor (VEGF)-VEGF receptor pathway (1). However, despite encouraging signs from some early preclinical studies that prolonged benefits would be seen in cancer patients, recent findings from the laboratory and clinic have uncovered several limitations to antiangiogenic therapy, posing future challenges for their expanding use. Currently approved antiangiogenic drugs include bevacizumab, the humanized monoclonal antibody to VEGF, as well as small molecule receptor tyrosine kinase inhibitors (RTKIs), such as sorafenib and sunitinib, which target VEGF and platelet-derived growth factor (PDGF) receptors (among a number of others). The VEGF RTKIs (approved thus far as single agents) and bevacizumab (approved for use only in combination with cytotoxic chemotherapy) can lead to disease stabilization and longer periods of progression free survival (PFS) or overall survival (OS) in many patients with metastatic disease, including colorectal carcinoma (CRC), metastatic breast carcinoma (MBC), non-small cell lung carcinoma (NSCLC), renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), gastrointestinal stromal tumors (GIST), and perhaps (though this has yet to be proven) in glioblastoma (GBM; reviewed in ref. 2). But tumors eventually become nonresponsive, or do not respond at all, despite the presence of VEGF and VEGFR-2, and PFS or OS in patients receiving antiangiogenic therapy has translated into benefits measured only in months, in most cases (3). Furthermore, in certain instances, increases in response rate and PFS do not always translate into increased OS for patients, as observed after bevacizumab treatment in RCC (as a single agent; ref. 4) or in MBC (in combination with a taxane chemotherapy; ref. 5). It also remains unclear what role drug combinations play in the efficacy of VEGF pathway targeting (antiangiogenic) inhibitors and why, at least to date, bevacizumab has proved largely ineffective as a single agent whereas VEGF RTKIs, with one recent exception (6), have repeatedly failed in randomized phase III trials when used in combination with chemotherapy (7).

Thus there is a growing interest in understanding the mechanisms of resistance, whether intrinsic or acquired, after exposure to antiangiogenic drug treatment. Early indications are that these mechanisms may be highly diverse, perhaps in part due to the primary mode of action of such drugs, e.g., blocking “host” tumor-supporting processes rather than blocking tumor growth directly. It is possible that resistance to antiangiogenic therapy may extend beyond classical drug resistance seen with traditional cytotoxic chemotherapy and radiation, or even molecular “tumor”-targeted therapy, which include rapid mutability and adaptability inherent to the tumor cells' genetic instability (ref. 8, see review ref. 9). Indeed an emerging question is whether the theoretical advantages of disrupting “host” angiogenic processes may be countered by significant disadvantages, including host-mediated resistance mechanisms involving the vascular microenvironment (perhaps largely independent of the tumor), as well as an altogether more disquieting possibility, namely, that antiangiogenic resistance may, in some instances, eventually increase or induce the invasive and metastatic potential of tumors as a result of therapy.

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The focus of this review is to discuss two interrelated pathways. The first includes the proposed main pathways of resistance to antiangiogenic therapy, differentiating between those mediated by the tumor itself or by the host (or both). The second pathway looks at disease progression from a localized primary tumor to established metastatic disease. It may be critical to consider both pathways simultaneously to understand and overcome some of these challenges facing antiangiogenic therapy, including mechanisms of drug resistance and how they may play a significant role in influencing tumor growth, for better or worse, at various stages of disease (Fig. 1).

**Resistance to VEGF Pathway-Targeting Agents**

*Fig. 1.* Mechanisms of resistance to antiangiogenic therapy may allow for differential efficacy in different stages of disease progression. Tumor-mediated mechanisms, either intrinsic or acquired by primary or established metastatic tumors, allow for evasion of anti-VEGF pathway inhibition. These mechanisms include: (1) tumoral upregulation of compensatory proangiogenic factors; (2) following initial tumor shrinkage, therapy-induced hypoxia mediated adaptation and/or evasion and tumor regrowth via rebound revascularization, vessel co-option, EMT, (3) increased invasiveness; (4) increased intravasal potential. Host-mediated mechanisms, either direct (in response to drug action) or indirect (in response to cues from the tumor), could include (5) BMDC mobilization; (6) microenvironment and/or stromal cell activation; (7) EC dysfunction, inflammation, and/or thrombosis; (8) pericyte dysfunction. Steps 5 through 7 could similarly lead to upregulation of compensatory proangiogenic molecules. Taken together, antiangiogenic therapy may increase tumor extravasal ability (9) and facilitate and/or accelerate disease in microscopic tumors. Legend: EC, endothelial cell; BM, basement membrane; BMDC, bone marrow-derived cell; CAM, compensatory angiogenic molecules; TAFs, tumor-associated fibroblasts; EMT, epithelial-mesenchymal transition.
in low oxygen environments (refs. 14, 15, recently reviewed in ref. 16).

Although resistance to VEGF pathway-targeted therapy may be mediated in large part by these intrinsic or acquired characteristics of the tumor cells, it is increasingly clear that the mechanisms involving the tumor microenvironment—either directly (in response to drug action) or indirectly (in response to cues from the tumor)—can also be involved in mediating eventual tumor relapse and regrowth. For example, stromal cells such as tumor associated fibroblasts (TAFs) can upregulate PDGF-C in response to VEGF inhibition (17). Pericytes could also play a role by retaining vascular function following endothelial cell (EC) disruption (18), regulating EC proliferation (9, 19), and/or providing a scaffold (along with remaining basement membrane-associated cells) for rapid revascularization after cessation of therapy (20). Moreover, various types of proangiogenic bone marrow-derived cells (BMDCs) may home to the tumor microenvironment and mediate resistance to VEGF pathway blockade via the production of the aforementioned compensatory proangiogenic factors (3). Examples include circulating cells such as Gr1+/CD11b+ myeloid suppressor-type cells (21) via Bv8 (prokineticin) and G-CSF-dependent mechanisms (22), TIE2-expressing monocytes via upregulation of angiopoietin-2 (23), and tumor-associated macrophages (also via upregulation of Bv8; ref. 24), and there are likely others (25). Taken together, these tumor- and host-mediated mechanisms, either alone or together in concert, may diminish response to antiangiogenic agents despite continued therapy. But what are the phenotypic characteristics of tumors that progress after initial benefit with antiangiogenic therapy?

Implications for tumor invasion and metastasis. The progression of a locally growing primary tumor to the growth of distant metastases involves a number of steps, including a loss of cellular adhesion; augmented motility and invasion capabilities; intravasation into the bloodstream; homing and survival; extravasation and seeding of micrometastases; and finally colonization and growth in a new distant site (26). Because of the integral role of the vasculature in this process, one obvious theoretical advantage of antiangiogenic therapy would be that some of these steps may be compromised, particularly in primary tumors (e.g., via the destruction of the immature vasculature to prevent and/or suppress intravasation), as well as in distant sites (e.g., the prevention of the "angiogenic switch" in avascular metastases). To date, extensive preclinical and clinical studies using VEGF pathway-targeting drugs have indeed been shown to stop or slow the growth of localized primary tumors or established metastatic disease, but it remains largely unknown how effective antiangiogenic therapy is at blocking earlier stages of metastatic disease. Clues that antiangiogenic agents may not sufficiently suppress metastasis in many cases and, even more provocatively, possibly select for more invasive and metastatic tumor phenotypes, have recently emerged. For example, in preclinical tumor models of GBM in which VEGF or HIF1α was genetically or therapeutically blocked (10, 27, 28), tumors initially shrank, but elevated hypoxia in the tumor microenvironment eventually caused or facilitated recurrent tumor growth in existing and adjacent sites. Moreover, Paz-Ribes and colleagues recently showed that therapy with anti-VEGFR antibodies or various VEGF RTKIs in genetically engineered RIP1-Tag2 pancreatic islet cell tumors and in orthotopically transplanted GBMs eventually resulted in tumors capable of increased invasion and metastasis in distant organs (such as the liver; ref. 29). Thus, in response to hypoxia induced by anti-VEGFR pathway-targeted therapy, tumors may acquire adaptive and/or evasive behavior. Interestingly, tumor-independent (host-mediated) pathways of resistance to angiogenesis inhibition may also facilitate tumor growth and metastasis in certain instances. For example, following short-term (7-day) treatment with various VEGF RTKIs in mice prior to intravenous inoculation of human tumor cells, or immediately after removal of a primary tumor, accelerated metastasis could be observed concomitantly with decreased survival (30).

But how can this be explained? Many possible mechanisms could be involved. For example, therapy-induced increases in tumor hypoxia and HIF1α expression following VEGF-pathway inhibition can lead to (i) increased c-met expression (31, 32) or IL6 expression (33); (ii) activation and/or upregulation of various matrix metalloproteinases (34); (iii) mobilization of BMDCs (35); (iv) instigation of tumor epithelial-to-mesenchymal transition (EMT) (ref. 36); all of which could increase invasive and/or metastatic potential in a "tumor-mediated" manner. Tumor-independent ("host-mediated") mechanisms could contribute as well, for example, via aforementioned therapy-induced upregulation of various proangiogenic molecules—many of which may increase the invasive and/or metastatic potential of cancer cells. For instance, it is now well documented that increases in VEGF and PIGF, and decreases in sVEGFR-2, can be observed in the plasma of patients receiving VEGF RTKIs (including sorafenib, sunitinib, and many others), such that it can be considered a "class effect" for these agents (see Supplementary Table 1 in ref. 37). Indeed this observation is a major reason why these proteins are currently being evaluated for use as potential surrogate biomarkers for tumor response (38, 39). However, many of these changes could derive, at least in part, from a systemic host-mediated response to treatment rather than from the tumor itself. This possibility was raised in the course of recent experiments from our laboratory that showed that dose-dependent, reversible elevations in VEGF/PIGF (and decreases in sVEGFR-2) could be recapitated in healthy tumor-free mice treated with VEGF RTKIs and could include many "off-target" molecules such as osteopontin, G-CSF, and SDF1α (13, 37). Given that many of these circulating proangiogenic cytokines, chemokines, and growth factors have been implicated in promoting angiogenesis and/or metastasis (40–44), it is theoretically possible that they assist in the aforementioned rebound revascularization (20), and/or increased extravasative potential for circulating tumor cells. Such induced systemic host responses to antiangiogenic drugs could facilitate an enhanced "premetastatic niche" precipitated by mechanisms largely independent of the tumor (30, 45). These include: (i) BMDC mobilization, such as the recruitment of circulating VEGFR-1+ bone marrow cells to distant organ sites (46, 47); (ii) pericyte dysfunction, which may in turn make vessels less mature and leaky, and allow for increased extravasative and/or metastatic tumor potential (48); (iii) increased prothrombotic events, which may be caused directly or indirectly by vessel damage as a result of therapy and allow for increased tumor cell "seeding" and growth in distant organs (49); (iv) altered EC adhesion molecule function, a possibility that was raised in a recent study that showed that inhibitors of α5β1/α3β1 administered continuously at low doses can enhance VEGF-driven angiogenesis and tumor growth (50); and (v) inflammatory pathway activation, which may lead to...
alterations (or injury) to the endothelial microenvironment, collectively increasing both intra- and extravasative potential for tumor cells (51). Thus both tumor- and host-mediated responses to antiangiogenic therapy, at least in certain instances, can facilitate proinvasive and metastatic potential after treatment in early-stage micrometastatic disease.

Although eventual enhancement of metastasis in response to an anticancer therapy may, at first glance, seem a counterintuitive concept (irrespective of whether mediated by tumor- or host-related mechanisms), it is important to note that similar findings have been reported for more than 30 years with cytotoxic treatments, including radiation and various chemotherapeutic drugs (52–54). Of course a presumed difference is that chemotherapy and radiation treatments act mainly by direct tumor cytotoxicity, e.g., by nonspecifically targeting proliferating cells, whereas antiangiogenic agents primarily target host processes. Furthermore, cytotoxic (and toxic) chemotherapy and radiation are administered for defined periods, e.g., 3 to 6 months, whereas antiangiogenic agents are (at least theoretically) meant to be administered for longer periods of time, if not indefinitely.

Clinical-Translational Advances

Is there clinical evidence of increased invasion and/or metastasis after VEGF pathway-targeted therapy? Although many host- and tumor-mediated pathways of resistance may explain, at least in part, some of the modest benefits attained in the majority of patients treated with anti-VEGF pathway-targeted agents, it remains unclear whether antiangiogenic therapy will lead to increased invasion and/or metastasis after either prolonged or short-term treatments in the clinic. To date, the literature surrounding this point remains largely anecdotal and limited to case reports or small studies, but there are some clues that suggest relapsed tumors may have an altered and/or increased progression after therapy stops working and/or when therapy is halted altogether. For example, in many instances human GBM patients treated with bevacizumab in combination with chemotherapy experience eventual tumor relapse and/or regrowth accompanied by a high rate of diffuse infiltrative lesions (55–59)—a finding suggestive of an adaptive-evasive response to antiangiogenic therapy leading to increased invasiveness. There are also instances in which discontinuation of anti-VEGF pathway-targeted therapy may support preclinical observations of “rebound revascularization,” something that in turn could influence tumor regrowth and/or metastasis. For example, cases of tumor “flares” have been reported during drug-free break periods (60), after treatment discontinuation in RCC patients receiving either sunitinib or sorafenib (61, 62), or in CRC patients treated with bevacizumab in combination with chemotherapy (63). Furthermore, in addition to primary tumor regrowth after treatment cessation with various VEGF RTKIs, increases in local foci or metastatic spread in distant organs have been noted in certain retrospective analyses (64). Importantly, there are emerging clues that some patients having failed to respond to (or been taken off) VEGF RTKI treatment, may respond again with the same drug after a break period (64), or respond when the drug is switched for another (e.g., sunitinib to sorafenib or vice versa) (refs. 65, 66).

Antiangiogenic therapy and micrometastatic disease: implications for adjuvant therapy? To date, one of the difficulties in uncovering (and predicting) antiangiogenic drug resistance mechanisms is a general disconnect between how such drugs are evaluated in experimental and clinical settings. For example, in most cases, patients in early phase clinical trials receiving antiangiogenic agents (or any other type of anticancer drug and/or therapy for that matter) have late-stage metastatic disease, often in multiple sites, whereas the majority of preclinical work focuses on localized primary tumors (67). Thus, it is essential that future testing of antiangiogenic (and other) therapies address this gap by investigating anticancer agents during various stages of tumor progression, especially when advanced metastatic disease is already established, or, conversely, when only microscopic metastases are present. These considerations are of particular relevance because (i) metastasis is generally the main reason for patient mortality rather than primary tumor growth (68), and (ii) antiangiogenic agents are now being evaluated in earlier stages of disease such as in the adjuvant setting, which may involve neither primary tumors or established metastasis, but rather early-stage occult micrometastatic disease. Indeed, surprisingly few preclinical studies have tested anti-VEGF targeted pathway drugs in early (micro) and late (established) stage metastasis, and even fewer still have directly compared antitumor efficacy in these indications to locally grown primary tumors. In such cases, mixed results have been observed, some of which may help explain modest clinical efficacies. For instance, VEGF RTKIs generally have been shown to slow or stop primary tumor growth in mice, but the effects on established metastatic disease range from efficacious (69), to only marginal or negligible benefit on the overall survival of mice (71). Moreover, in micrometastatic and/or early stage disease, the aforementioned studies by our and the Paez-Ribes and colleagues’ groups show that VEGF pathway-targeted therapy can, in certain instances, result in increased tumor invasiveness and metastasis. Critically, such results contrasted in both studies with the potent tumor growth inhibitory effects the same drugs and treatment schedules had on locally grown primary tumors (29, 30). It is likely that various experimental conditions—such as the animal model, tumors, drugs, doses, treatment duration, or combinations with chemotherapy—may explain some of these differences in experimental outcomes; however, it is possible that differential efficacies with antiangiogenic therapy may be observed between micro- and macrometastatic disease. Some studies with genetically engineered mouse models of intestinal adenomas (APCmin mice) show that tumor growth can be slowed and survival prolonged after treatment with various inhibitors of the VEGF pathway (72–74) Similarly, transgenic mouse models of NSCLC, generated by mutations in Kras showed improved survival in mice treated with sunitinib starting 4 weeks after metastatic tumor growth was induced by conditional Lkb1 deletion. Yet, outcome in this latter instance was not further improved if treatment started 2 weeks earlier (75, 76), raising the question whether observed benefits after treatment were because of effects against established metastasis rather than microscopic disease.

Such distinctions could be important for interpretation of both preclinical and clinical trials involving antiangiogenic agents in the adjuvant setting (77). In limited preclinical models in which primary tumors are removed and antiangiogenic drug treatment is initiated, metastatic growth could be inhibited (69, 78) or accelerated (30), depending on the tumor models and drugs used, and when treatments are initiated. It is clear that more studies should be conducted preclinically to test anti-VEGF
pathway-targeted therapy in an authentic adjuvant setting, i.e., very shortly or immediately after surgical resection of a primary tumor when only microscopic minimum residual disease is present—something that might be determined by various imaging techniques and/or other measures (79). Ironically, it might be that this question will be addressed and answered first in the clinical setting. Currently there are more than 40+ adjuvant clinical trials underway involving multiple VEGF pathway inhibitors, such as sorafenib and sunitinib (80), as well as bevacizumab (typically in combination with chemotherapy), in numerous cancer types, including breast, renal, prostate, head and neck cancers, NSCLC, ovarian, and others.\textsuperscript{1} With respect to bevacizumab, one such trial has been completed as of 2009. In this phase III study, postoperative colorectal patients with stage II–III disease were treated with the anti-VEGF antibody plus chemotherapy for 1 year and 6 months, respectively. The results of this trial\textsuperscript{2} showed no benefit in PFS when assessed 3 years after therapy initiation (81). Interestingly, a clear benefit in favor of this trial showed no benefit in PFS when assessed 3 years after therapy for 1 year and 6 months, respectively. The results of this phase III study, postoperative colorectal patients with stage II–III disease were treated with the anti-VEGF antibody plus chemotherapy for 1 year and 6 months, respectively. The results of this trial showed no benefit in PFS when assessed 3 years after therapy initiation (81). Interestingly, a clear benefit in favor of bevacizumab at 1 year (when therapy was completed) was observed, but this benefit gradually disappeared over time. The basis for this phenomenon is unknown and clearly highlights the urgent need for undertaking preclinical studies in appropriate models to examine the mechanisms by which antiangiogenic treatments such as VEGF pathway-targeted drugs lose their activity and/or alter tumor progression and metastasis over time.

Conclusion

Although anti-VEGF pathway-targeted therapies represent an effective treatment of cancer in certain settings, substantial benefits remain unrealized for the vast majority of patients in terms of overall survival. Preclinical testing has uncovered mechanisms of resistance that may, in certain instances, be differentiated between host- and tumor-mediated processes, some of which could explain limitations seen with the use of antiangiogenic drugs in the clinical setting. Further understanding of these mechanisms, as well as consideration of the potential differential efficacies of treatments at different stages of tumor progression, will be key to devising rational strategies in overcoming the challenges facing further development of antiangiogenic therapies.

Disclosure of Potential Conflicts of Interest

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