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Minireview

Silencing or Fueling Metastasis with VEGF Inhibitors: Antiangiogenesis Revisited

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Clinical practice reveals that therapy with angiogenesis inhibitors often does not prolong survival of cancer patients for more than months, because tumors elicit evasive resistance. In this issue of *Cancer Cell*, two papers report that VEGF inhibitors reduce primary tumor growth but promote tumor invasiveness and metastasis. These perplexing findings help to explain resistance to these drugs but raise pertinent questions of how to best treat cancer patients with antiangiogenic medicine in the future. We discuss here how VEGF inhibitors can induce such divergent effects on primary tumor growth and metastasis.

Rooted in the belief that blocking vessel supply starves tumors to death (Folkman, 1971), it has become increasingly accepted that blocking tumor angiogenesis as much as possible would provide cancer patients maximal survival benefit. Given the key importance of VEGF and its receptor VEGFR2 in angiogenesis, hopes were raised that blocking this pathway would eradicate the tumor vasculature and heal cancer. Indeed, the monoclonal anti-VEGF antibody bevacizumab (Hurwitz et al., 2004; Miller et al., 2007) and the second-generation multitargeted receptor tyrosine kinase inhibitors (RTKIs) sunitinib (Demetri et al., 2006; Motzer et al., 2006) and sorafenib (Abou-Alfa et al., 2006; Escudier et al., 2007) have prolonged the life of numerous cancer patients. These successes have revolutionized the face of clinical oncology. However, clinical experience has also revealed that VEGF-targeted therapy often prolongs overall survival of cancer patients by only months, without offering enduring cure (Kerbel, 2008). In this issue of *Cancer Cell*, two leading angiogenesis laboratories present intriguing, almost perplexing evidence that VEGF-targeted drugs inhibit primary tumor growth yet may shorten survival of mice by promoting tumor invasiveness and metastasis (Ebos et al., 2009; Pàez-Ribes et al., 2009). These findings help to explain evasive resistance to these drugs but also raise a number of pertinent questions of how to best combat cancer with antiangiogenic medicine in the future.

In most cases, metastasis, not primary tumor growth, kills the cancer patient (Gupta and Massague, 2006). Yet in the past, the majority of preclinical studies have focused on probing how antiangiogenic drugs inhibit primary tumor growth, with less attention on metastasis. Consistent with previous findings, Ebos et al. (2009) and Pàez-Ribes et al. (2009) report that different classes of VEGF-targeted therapies (e.g., VEGF RTKIs or anti-VEGFR2) or *VEGF* gene inactivation in tumor cells inhibits primary tumor growth in various cancer mouse models and, in some cases, provides a survival benefit. Strikingly, however, pretreatment of healthy mice with these VEGF inhibitors prior to intravenous inoculation of tumor cells "conditioned" them to more aggressive metastasis with shortened survival. Also, adjuvant short-term VEGF RTKI treatment after resection of the primary tumor enhanced spontaneous metastatic tumor burden. Furthermore, brief treatment of spontaneous and orthotopic tumor models with VEGF inhibitors caused a persistent switch to "vasoinvasion" of tumor cells, leading to increased metastasis. At first sight, these findings are inconsistent with previous observations that anti-VEGF reduces metastasis and are difficult to reconcile with the prevailing dogma that tumors, primary or metastatic, require vessel supply for growth (discussed in Crawford and Ferrara, 2009). How can we explain these divergent effects of VEGF-targeted therapy? Figure 1 highlights a number of possible reasons.

One plausible mechanism is tumor hypoxia. The more proficient an antiangiogenic agent is, the more efficiently it will prune tumor vessels and hence cause hypoxia. Unlike normal cells, tumor cells are much better equipped to cope with hypoxia (Brahimi-Horn et al., 2007). Apart from metabolic reprogramming to glucose addiction, which allows tumor cells to generate energy in hypoxic conditions, hypoxia-tolerant tumor cell clones are selected, while tumor stem cells in hypoxic niches escape antiangiogenic treatment (Brahimi-Horn et al., 2007). Hypoxia thus selects for more malignant metastatic cells, which are less sensitive to antiangiogenic treatment (Yu et al., 2002). In support of this concept, treatment of mice with anti-VEGFR2 induces a shift in glioblastoma tumor phenotype toward enhanced migration and invasion (Kunkel et al., 2001). In addition, tumors recruit other vascular supply mechanisms, such as mobilization of angiocompetent bone marrow-derived cells (Grunewald et al., 2006) or co-option of existing vasculature, that are not always inhibited by VEGF-targeted therapy (Bergers and Hanahan, 2008). But tumor cells also are proficient in escaping the noxious hypoxic microenvironment by switching on invasive epithelial-mesenchymal transition (EMT) and other metastatic programs (Brahimi-Horn et al., 2007). Recent evidence indicates that this metastatic switch is incited by narrow changes in tumor oxygen levels (Mazzone et al., 2009). Hence, unlike normal cells, tumor cells tolerate hypoxia better but at the same time also more vigorously escape hypoxia.

Ebos et al. (2009) show that pretreatment of healthy (non-tumor-bearing) mice with VEGF inhibitors prior to intravenous

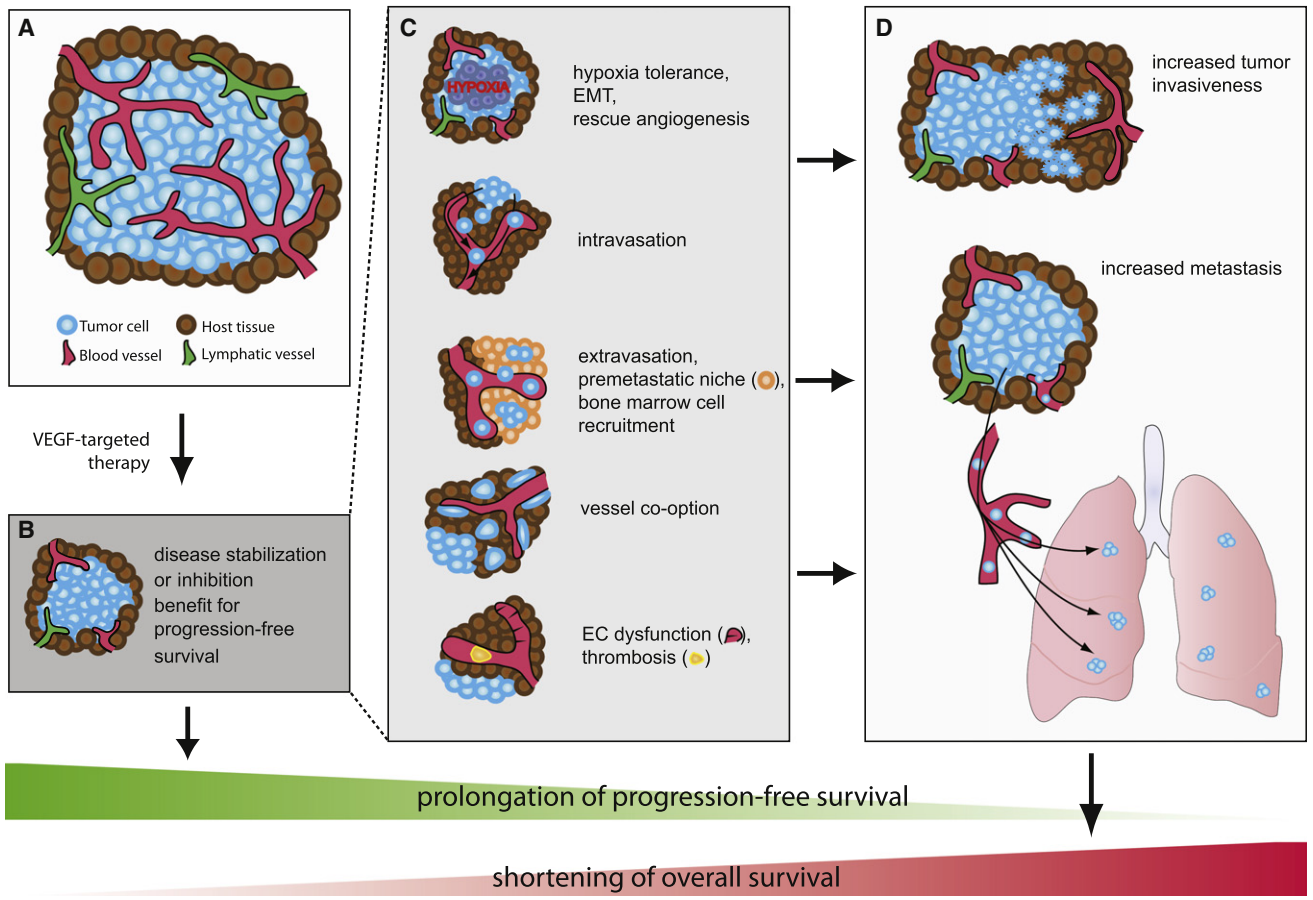

Figure 1. Increased Tumor Invasiveness and Metastasis Evoked by VEGF Inhibitors
(A) Tumors need blood (red) and lymphatic (green) vessels to grow.
(B and C) VEGF-targeted therapies induce primary tumor shrinkage and inhibit tumor progression but can also initiate mechanisms that increase malignancy. EMT, epithelial-mesenchymal transition; EC, endothelial cell.
(D) As result, anti-VEGF treatment can enhance tumor invasiveness and metastasis and reduce overall survival benefit.

injection of tumor cells also promotes metastasis. While the underlying mechanisms remain to be explored, effects on tumor cell extravasation or formation of a premetastatic niche should be considered, to name just a few. For instance, VEGF inhibitors are known to prune quiescent vessels in healthy tissues, which upon withdrawal of therapy show a rapid rebound growth; such a well-vascularized niche may promote seeding of metastasizing tumor cells (Kamba et al., 2006). In addition, even in healthy mice, these VEGF inhibitors dose-dependently induce a chronic "inflamed" state characterized by elevated levels of G-CSF, SDF1α, PlGF, SCF, IL-6, erythropoietin, osteopontin, and other cytokines that stimulate metastasis and angiogenesis in a VEGF-independent manner (Ebos et al., 2007); some of these cytokines also recruit angiogenic bone marrow-derived endothelial and myeloid progenitors that promote the formation of a premetastatic niche (Kaplan et al., 2005). Certain subclasses of these cells only express VEGFR1, and their recruitment will therefore not be blocked by VEGF inhibitors with a restricted profile (such as anti-VEGFR2) (Kaplan et al., 2005). Perhaps these cytokines "inflame" the endothelium, thereby facilitating adhesion, permeability, and egress of tumor cells from the vasculature (Gupta and Massague, 2006). Because VEGF is a survival factor for endothelial cells, its inhibition can cause vessel disintegration and render the endothelium prothrombotic, which could also affect tumor cell lodging (Lee et al., 2007).

There could be other mechanisms that underlie, or at least fail to counteract, metastasis in VEGF inhibitor-treated mice. For instance, some of these agents (especially the more broad-spectrum VEGF RTKIs, which also inhibit PDGFRβ) or the cytokine response they induce (in the case of osteopontin) may inhibit coverage of tumor vessels by pericytes, which destabilizes vessels, makes them more leaky and immature, and hence facilitates intravasation of tumor cells and metastasis (Bergers and Hanahan, 2008). Another mechanism involves vessel co-option, whereby tumor cells ensheathe preexisting vessels and travel alongside "oxygen pipes" to invade healthy tissue (Bergers and Hanahan, 2008). This mechanism may be more relevant in particular tumors, such as in the brain.

Are the preclinical findings by Ebos et al. and Pàez-Ribes et al. of general importance? Previous studies have documented that VEGF-targeted therapy inhibits primary tumor growth and metastasis (Sini et al., 2008; Verheul et al., 2007). In several of these mouse models, metastasis correlates closely with tumor burden—i.e., the bigger the tumor, the more malignant cells

can escape and hence the larger the metastatic burden. Metastasis is also inhibited by anti-VEGF after resection of primary tumors (Mizobe et al., 2008). However, others have observed no inhibition or only negligible effects of VEGF inhibitors on metastasis (Francia et al., 2008). The reasons for these discrepancies remain speculative, but, based on the mechanisms postulated above, one can hypothesize that quantitative and qualitative effects of VEGF-targeted therapy may depend on a number of variables. These may include tumor-intrinsic parameters such as VEGF levels, vessel number and function, sprouting angiogenesis versus vessel co-option, VEGF dependence of tumor vasculature, pericyte coverage, recruitment of bone marrow cells, oxygen tension, lymphatic versus hematogenous metastasis, etc. or may involve differences in experimental conditions such as type of tumor and metastasis model, duration of treatment, class and dose of VEGF inhibitor, monotherapy versus combination treatment with cytotoxic or other agents, etc. (see above). Pàez-Ribes et al. speculate that high VEGF levels render tumor vessels immature and facilitate intravasation and metastasis while inhibiting VEGF may produce a similar overall effect on metastasis, though via qualitatively distinct mechanisms, as explained above. Another unresolved issue is whether metastatic tumors rely as much on angiogenesis as primary tumors, given that metastatic tumor cells often acquire hypoxia-tolerant properties (Brahimi-Horn et al., 2007).

Emerging clinical evidence is consistent with the findings of Ebos et al. and Pàez-Ribes et al. Indeed, the invasiveness and metastatic behavior of tumor cells after VEGF-targeted therapy may explain why, after a transitory period of primary tumor growth inhibition and prolongation of progression-free survival, clinical responses do not endure and tumors relapse as more invasive metastatic disease, thereby limiting the benefit for overall survival (Kerbel, 2008). Also, reports that induction of tumor hypoxia and the systemic ''inflamed'' state is dependent on the VEGF inhibitor dose could explain why ''more'' of a VEGF inhibitor, in terms of dose or potency, has not consistently been ''better'' for patient outcome, though this deserves further study (Cannistra, 2008). Furthermore, the observation that even brief treatment with VEGF inhibitors for only a few days suffices to induce persistent, irreversible alterations in tumor cell invasiveness may explain why rapid tumor regrowth has been observed in some cancer patients during short-term ''drug holidays'' (Batchelor et al., 2007).

These findings have pertinent implications for antiangiogenesis medicine and raise questions as to how they can best be translated to the clinic. For instance, are these findings class-specific for VEGF inhibitors alone, or do other antiangiogenic agents induce similar prometastatic effects? New antiangiogenic agents, such as Dll4 inhibitors, inhibit primary tumor growth but also cause hypoxia via formation of hypoperfused vessels (Noguera-Troise et al., 2006). VEGF inhibitors induce vessel normalization during a particular time window (Batchelor et al., 2007; Jain, 2005); since this process has been related to increased drug delivery, it has been questioned whether pretreatment of cancer patients with VEGF inhibitors would improve chemotherapy. Do the present findings suggest that the risk of metastasis associated with neoadjuvant VEGF-targeted therapy may outweigh the benefit of improved cytotoxic drug delivery? Will maintenance of VEGF-targeted therapy be beneficial by suppressing primary tumor growth, or will it be harmful by evoking evasive resistance? Can combination therapy with antimetastatic medicine overcome the prometastatic effects of VEGF inhibitors and turn them into more effective anticancer drugs? Intuitively, one might postulate that antiangiogenic treatment should be initiated as early as possible in cancer, but clinical evidence shows that, in metastatic colorectal cancer patients, continuation of VEGF-targeted therapy even beyond disease progression still improves clinical outcome (Grothey et al., 2008). Future clinical studies are thus warranted to assess the relative risk of metastasis versus the benefit of tumor starvation in cancer patients and whether this differs for curable nonmetastatic versus advanced metastatic disease. Indeed, a recent report indicates a higher incidence of distant recurrence of glioblastoma in patients treated with the anti-VEGF antibody bevacizumab and chemotherapy than in patients treated with chemotherapy alone, but overall, patients treated with the combination therapy still lived longer (Zuniga et al., 2009).

Another question is whether combining antiangiogenic drugs with other classes of targeted agents, such as inhibitors of the prometastatic scatter factor/HGF receptor MET or of epidermal growth factor receptor signaling, might help to counteract the switch to more aggressive behavior elicited by VEGF inhibitors. However, a recent study suggests that, contrary to predictions by preclinical research (Stommel et al., 2007), simultaneous inhibition of the VEGF and EGF pathways in combination with chemotherapy shortens rather than prolongs progression-free survival as compared to inhibition of the VEGF pathway alone in combination with chemotherapy (Tol et al., 2009). It thus remains to be determined whether other targeted agents exhibit beneficial effects when combined with VEGF inhibitors. Moreover, development of reliable biomarkers to monitor development of evasive resistance to antiangiogenic drugs could render this therapy more effective (Jain, 2008). In this respect, genetic polymorphisms in the *VEGF* gene were recently found to be associated with response to anti-VEGF treatment (Schneider et al., 2008). Hopefully, such strategies may help to identify individuals at greater risk for these undesirable adverse effects of VEGF-targeted therapy and lead the way to possible future tailoring of individualized antiangiogenic therapy.

Finally, are there any alternative antiangiogenic agents that might not evoke this malignant behavior? Given that enhanced tumor invasiveness and malignancy appear to be more severe upon complete blockade of VEGF, could a combination or substitution of VEGF inhibitors with agents that induce less hypoxia (such as anti-PlGF [Fischer et al., 2007]) provide an alternative? Or should conceptually distinct antivascular strategies be considered? For instance, can tumor vessel function, rather than numbers, be targeted? A recent study shows that haplodeficiency of *PHD2* in endothelial cells improves tumor vessel perfusion via normalization of their endothelial layer and thereby shifts the tumor to more benign, less invasive and metastatic behavior (Mazzone et al., 2009), which is also more responsive to chemotherapy (unpublished data).

In the ancient Greek myth, when Odysseus had to choose between two life-threatening evils, Scylla and Charybdis, he managed to avoid both. Future studies will be required to develop strategies that will allow us to optimally exploit the potential of VEGF inhibitors to block primary tumor growth while

at the same time suppressing prometastatic effects, without having to choose between the evils of increased primary tumor growth in untreated conditions and induction of metastasis in treated conditions.

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