Building a better Trap

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A wide variety of antiangiogenic agents are now being tested in late-stage cancer patients as stand-alone agents or in combination with standard therapy (1). Although there have been mixed reviews so far, a success story appears to be emerging (2). In a recently concluded Phase III clinical trial involving patients with advanced colon cancer, Avastin [antivascular endothelial growth factor (VEGF)], in combination with chemotherapy, showed remarkable antitumor activity. These exciting clinical data breathe new life into the angiogenesis field and provide hope that Avastin and other agents now under clinical evaluation will ultimately become components of standard therapy for cancer and perhaps other diseases associated with angiogenesis. In a recent issue of PNAS, Huang *et al*. (3) describe a new and perhaps more effective approach to block tumor-associated VEGF. By using a high-affinity anti-VEGF therapy, VEGF-Trap (4), the investigators report the regression of large preexisting primary and metastatic tumor xenographs. By using a better or higher-affinity VEGF inhibitor, the investigators claim that VEGF levels are removed to such a degree that even preexisting tumor vessels can be destroyed. Thus, it is concluded that potent blockade of VEGF may provide a new therapeutic option for patients with bulky metastatic cancers.

To grow and metastasize, tumors must stimulate the development of new vasculature through a process known as angiogenesis (5). Angiogenesis is a dynamic progression that begins with endothelial sprouting from a preexisting blood vessel and ends with the establishment of a mature vascular plexus (6). Much of the maturation of the vascular plexus occurs when mural cells such as pericytes and vascular smooth muscle cells migrate to newly formed loops of endothelial cells, sheath them, stabilize them, and thereby form arterioles, capillaries, and veins (7). This process occurs normally whenever there is a significant increase in tissue mass such as in development, growth of the corpus luteum, or accumulation of adipose tissue. However, during tumor growth, blood vessels frequently remain in a dynamically remodeling, chaotic, irregular, and leaky state with abnormal infiltration by mural cells long after endothelial loops are established (8).

The distinctive properties of tumor blood vessels have made them an enticing target for tumor therapies. Although conventional therapies target neoplastic cells within a tumor, antiangiogenic therapy offers several potential advantages as an approach to cancer treatment, notably physical accessibility and genetic stability of target cells (5). Because of these advantages, several angiogenesis inhibitors have progressed to clinical trial stage, including both peptide (9) and antibody (10) integrin antagonists, antiangiogenic proteins such as endostatin (11), matrix metaloproteinase inhibitors (12), and inhibitors of angiogenic growth factors (13). Of these, VEGF, a central mediator of angiogenesis, has successfully emerged first from the clinic as an important target for antiangiogenic therapy. VEGF is an endothelial cell-selective mitogen that appears to be required for the survival of immature blood vessels (14). Its gene expression is up-regulated by starvation, growth factors, and hypoxia and is found in numerous tumors. Inhibitors of VEGF, including small molecules, monoclonal antibodies, and soluble receptors have been generated and shown to be effective at inhibiting the growth of tumors *in vivo* (15, 16).

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Clinically, VEGF inhibitors are currently undergoing evaluation of numerous cancer indications for effects on patient survival and time to progression alone and in combination with chemotherapeutics (14). Although initial trials in breast cancer failed to show significant improvement in clinical end-points, recent trials evaluating the effects of anti-VEGF therapy in combination with chemotherapeutic against metastatic colorectal carcinoma have dramatically improved patient survival and time to progression. A key question is: Other than indications evaluated, what are the differences between the two trials that could have led to the disparate trial outcomes? One possibility brought forth is that the breast cancer trials evaluated patients in late-stage disease when the tumor burden was large, whereas the colorectal trial evaluated patients in earlier stages. This result tracks well with preclinical results indicating that anti-VEGF therapy is more effective in earlier than later stages of disease (17). However, preclinical evidence indicates that other antiangiogenic modalities are effective against large later-stage tumors (18–19), suggesting that the limitations seen to date with anti-VEGF therapy are not a general limitation of antiangiogenic therapy. A remaining question then becomes whether the failure of anti-VEGF therapy against late-stage disease is a limitation of anti-VEGF therapy or of the VEGF inhibitors used.

In their article, Huang *et al*. (3) propose that anti-VEGF therapy can be successful against late-stage disease if the therapeutic modality is potent enough. The authors used an innovative anti-VEGF therapy coined VEGF-Trap in which Ig domains from the lower-affinity VEGF receptor Flk and the high-affinity VEGF receptor Flt-1 are fused to generate a soluble VEGF inhibitor with favorable pharmacokinetic properties and an extraordinarily high binding affinity (kDa $\approx$1 pM) (4). Using this construct, they show dramatic (>80%) regression of large established human tumors and associated metastases in a xenograft model coincident with a progressive ablation of the tumor vasculature. These studies indicate for the first time that this next generation of anti-VEGF therapy has promise as a single agent in the treatment of bulky advanced-stage cancer.

Paradoxically, Huang *et al*. (3) indicate that, whereas VEGF-Trap did not impact mature normal vessels, it induced apoptosis in tumor vessels that had matured to the point of being associated with mural cells. This finding suggests that either tumor vessels are somehow distinct from the normal vasculature irrespective of the presence of perivascular stabilizing cells or the perivascular cells are abnormally associated with the endothelial cells. In fact, recent reports

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indicate that the latter may be the case (8). Furthermore, the therapy destabilized these vessels by inducing apoptosis of both the tumor endothelium and the associated perivascular cells. Although apoptosis of the mural cells might be expected due to reduced nutrient delivery from the vessels, Huang *et al.* report that the death was in a parallel time frame, beginning 1 d after delivery of VEGF-Trap. However, although VEGF has been implicated in the migration response of some stromal cells (20), VEGF alone has not been reported to have prosurvival benefits for perivascular cells. This finding suggests the interesting possibility that there is a co-dependent survival relationship in immature vessels between mural and endothelial cells that requires low levels of VEGF.

Another interpretation of the data, other than a novel capacity of the therapy to induce apoptosis in mature tumor vessels but not mature normal vessels, is that the therapy may strongly impact the remodeling vasculature within the tumor. The persistently high levels of growth factors within a tumor lead to a perpetually remodeling vasculature in which many tumor blood vessels are in a continual state of growth, regression, and regrowth (21). These vessels are important in the expansion and maintenance of a tumor, yet they are not completely mature and might be sensitive to a more potent inhibitor of VEGF (22). Yet another interpretation might be that the inhibitor is binding other growth factors that are required for maintenance and survival of both endothelial and mural cells in the more hostile microenvironments of a growing tumor.

Although the VEGF-Trap described was effective in a xenograph orthotopic model of a human Wilms tumor, it is not clear whether such findings will translate to human cancer. Xenograph tumors involving the injection of cultured human cells into nude mice do not mirror many of the events that take place in cancer patients. For example, human tumors develop spontaneously based on genetic mutation and grow over the span of months to years while gradually accessing a blood supply. In contrast, xenographs grow to a large size, develop a vascular supply within days of injection, and do not have to contend with much of a host immune response. It will be important to test the VEGF-Trap in spontaneous cancer models such as the Rip-Tag mouse (23) or other syngeneic models that more closely mirror the human disease. This assertion is based on the fact that several angiogenesis inhibitors, including those that target VEGF or its receptor, have performed exceptionally well in experimental mouse tumor models but have shown considerably less activity in human patients. It is conceivable that the use of spontaneous cancer models will allow investigators to better predict the outcome in humans.

Although Huang *et al.* (3) suggest they have built a better VEGF inhibitor, it will be important to compare their approach with other such inhibitors in the same physiologically relevant model. Such a comparison will allow one to conclude whether a given inhibitor has a particular benefit. As indicated above, it will be important to understand whether some tumors in general are more sensitive to a decreased blood supply or whether a particular organ or microenvironment is more sensitive to a given angiogenesis inhibitor. Answers to these questions will provide a rational basis for the development of potentially very active antiangiogenic agents that will ultimately be included as a standard therapeutic approach for the treatment of cancer patients.

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