

# Targeting Coagulation to the Tumor Microvasculature: Perspectives and Therapeutic Implications From Preclinical Studies

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Pioneering studies at the National Cancer Institute by Glenn Algire in 1945 led to the conclusion that “the rapid growth of tumor explants is dependent on the development of a rich vascular supply” (1). There is now little doubt that most tumors are dependent on neovascularization for oxygen and nutrients to sustain progressive growth (2). In 1971, Judah Folkman (3) proposed the innovative idea that angiogenesis inhibitors could be used to treat cancer. In 2004, the U.S. Food and Drug Administration approved the first antiangiogenic drug for the treatment of cancer, a humanized monoclonal antibody against VEGF-A named Avastin (bevacizumab). Other drugs in this category are under development.

There are at least two general approaches to depriving tumors of their blood supply. One approach is to inhibit tumor-induced neovascularization from both the sprouting neighboring vessels and the recruitment of endothelial cell precursors from the bone marrow (4–8). The essential role of vascular endothelial growth factor (VEGF) in this process is well established, and several angiogenesis inhibitors have shown efficacy at reducing tumor neovascularization and tumor growth, at least in preclinical models (9–11). In some cases, rapidly growing lesions were converted into dormant lesions (12). A second approach to reducing the tumor blood supply is to cause zonal tumor necrosis by targeting the preexisting vasculature (13,14). This approach is used successfully in the clinic, where transarterial embolization is used to obstruct vessels, particularly in hepatocellular carcinoma (15). Recently, randomized controlled trials have shown increased survival of patients treated in this way (16,17). As elegantly illustrated by Dienst et al. in this issue (18), recent advances in the molecular characterization of tumor endothelium

have permitted an evolution of this catheter-based approach to vessel occlusion.

The targeting of vascular endothelial cells presents several advantages compared with tumor cell targeting. First, effective and uniform delivery of anticancer drugs to solid tumors has proved challenging: Drugs do not reach deep beyond the perivascular region due to physical barriers by fibrous tumor tissue and to elevated interstitial pressure, which reduces fluid convection (19). By contrast, endothelial cells lining the vessels are easily accessible to the bloodstream. Second, vascular endothelial cells are less heterogeneous than tumor cells and, as generally normal, nontransformed cells, are less likely than tumor cells to acquire mutations leading to drug resistance (7). Third, complete cell death in all endothelial cells lining a vessel is not necessary; rather, interruption of the blood stream for a period of hours may suffice.

At the same time, these advantages are the base of difficulties to overcome. The most critical point is specificity of tumor vascular targeting to avoid undesirable effects to normal tissue blood supply. Thus, selection of tumor-specific target molecules is the key to the success of this approach. Fortunately, tumor and normal tissue vasculature differ in morphology and function.

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That is, in contrast to normal tissue vessels, tumor vessels are leaky, twisty, and dilated; pericytes are irregularly covered; and basement membranes are abnormal (19). Recently, high-throughput techniques have permitted the identification of molecular “ZIP codes” that mark angiogenic tumor endothelial cells and distinguish them from nonreplicating endothelial cells. In particular, peptide sequences that bind preferentially to tumor endothelium have recently been identified from peptide phage display libraries. These sequences include Arg-Gly-Asp (RGD)-containing peptides, which have high affinity for  $\alpha_v$  integrins (20), as well as peptides directed to membrane-bound matrix metalloproteinases (21) or aminopeptidases (22). Moreover, serial analysis of gene expression methods have revealed some differences between normal vessels and those derived from colorectal, brain, lung, and liver tumors (23). Recently, proteomic and bioinformatic analyses of the endothelial cell surface have identified aminopeptidase-P and annexin A1 as selective molecular targets in solid tumor-associated vasculature (24). Furthermore, radioimmunotherapy against annexin A1 increased survival in preclinical studies (24). Peptides, antibodies, and natural ligands that bind the tumor endothelium with high degree of selectivity and affinity hold promise as potential anticancer drugs. Cocktails of reagents, which target different tumor-selective vascular markers, may help increase levels of drugs reaching the tumor vessels.

Once specific vascular targets are identified, several different effector molecules could be targeted to the tumor vasculature, including radioactive reagents (24), cytotoxic agents (25), and antiangiogenic and immunostimulatory cytokines, such as interleukin 12 (26). Another effector molecule is tissue factor (TF), a cell membrane receptor that initiates the extrinsic pathway of the blood coagulation cascade. The truncated extracellular domain of TF is five orders of magnitude less potent than the membrane form because the TF-factor VIIa complex requires negatively charged cell surface phospholipids to activate the downstream factors IX and X, which in turn catalyze the formation of thrombin (27). However, once bound to target tumor vessels, soluble TF initiates an explosive coagulation cascade that leads to intravascular thrombosis and tumor infarction (28).

In this issue of the Journal, Dienst et al. (18) evaluated a novel single-chain anti-VCAM-1 antibody/soluble TF fusion protein as a tumor vascular targeting agent. The authors observed convincing tumor tissue necrosis and intratumor vascular fibrin thrombosis following a single systemic administration of fusion protein. In addition, long-term treatment produced statistically significant delays in tumor growth, at least under certain conditions. Importantly, after extensive analyses, the authors could find no evidence for treatment-related increase in vascular thrombosis and necrosis in normal tissues and no evidence for systemic activation of the coagulation cascade. This work adds support to the notion that strategies aimed at producing vascular occlusion in tumor tissues can be effective and selective.

VCAM-1 is a type I transmembrane glycoprotein that is generally not expressed by normal endothelium but is induced by inflammation. The tumor vasculature and some tumor cells generally express VCAM-1. The authors observed that vascular expression levels of VCAM-1 differed in two distinct tumor models. Previously, vascular endothelial cells have been noted to have some tissue specificity (29,30). Recently, the endothelial cell-associated surface marker VE-cadherin has been reported to display some organ specificity (31), and genetic screening has

identified several genes that are expressed selectively in the normal lung and kidney endothelium but not in other normal endothelium (32), raising the possibility that at least some vascular markers may also be tumor type-specific. Now that several tumor vessel-associated markers have been identified, it will be important to establish which has the highest degree of tumor vessel specificity and whether there are differences among tumor types. Resolving these issues promises to bring an exciting, albeit complex, period of investigation to the field.

If vascular targeting approaches such as the one described by Dienst et al. (18) are developed into cancer therapeutics, they have the potential to add a powerful punch in the fight against cancer. Clearly, more work needs to be done, but the outlook is promising.

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