Antiangiogenesis: Vessel Regression, Vessel Normalization, or Both?

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The concepts of antiangiogenic tumor therapy were pioneered on the assumption that the inhibition of tumor angiogenesis should lead to the complete regression of the tumor-associated vasculature and thereby hold the tumor in an avascular dormant state. Yet, clinical trials revealed limited efficacy of angiogenesis inhibitors when used as monotherapy. Instead, antiangiogenic drugs proved effective to extend overall survival when used in combination with chemotherapy. This counterintuitive observation—inhibition of tumor vascularization should lead to less and not more delivery of chemotherapy to the tumor—led to the concepts of "vessel normalization." This refers to the notion that antiangiogenic drugs prune the most immature tumor vessels and spare mature vessels, thereby resulting in a more normal-appearing vasculature that leads to better access of chemotherapy to the tumor. The concepts of vessel normalization were first laid out in a landmark publication in *Cancer Research* in 2004. More than 600 studies on different aspects of vessel normalization have been published since then. Nevertheless, it is to this day less clear than ever to what extent vessel regression (leading to tumor starvation) and vessel normalization (facilitating chemotherapy) contribute to the clinical efficacy of antiangiogenic tumor therapy. This "Landmark Commentary" puts the concepts of tumor vessel normalization in historical context and develops thereupon some of the most burning questions in the field of translational angiogenesis research that need to be answered to further advance the application of tumor vascular stroma reprogramming therapies.

See related article by Tong and colleagues, Cancer Res 2004;64:3731–6.

It has been 50 years since the legendary *New England Journal of Medicine* article by Judah Folkman proposing the angiogenesis dependency of tumor growth (1). Folkman predicted that (i) tumors would be restricted to microscopic size in the absence of angiogenesis, (ii) suggested that tumors secrete diffusible angiogenic molecules, (iii) described a model of tumor dormancy due to blocked angiogenesis, (iv) proposed the term "antiangiogenesis" for the prevention of new capillary sprouts from being recruited into a growing tumor, (v) envisaged the future discovery of angiogenesis inhibitors, and (vi) proposed the idea that an antibody to a tumor angiogenic factor could be an anticancer drug (1). This visionary publication set the stage for modern-day angiogenesis research. What is so self-evident today, namely that tumor growth is angiogenesis dependent, was not readily accepted at the time. Instead, it was widely believed that tumors grew along preexisting capillaries, a process now known as "vessel co-option," which may contribute to tumor growth (2). Yet, it is nowadays absolutely undisputed that the progression of the vast majority of human tumors is critically dependent on neo-angiogenesis, the growth of new blood vessels originating from preexisting vessels.

Partly due to the skepticism against the bold hypotheses put forward by Folkman, it took almost 20 years until the discovery of the master regulator of the angiogenic cascade VEGFA (or VEGF) by Napoleone Ferrara in 1989 (3). Pleiotropically acting angiogenesis factors had been identified earlier, but VEGF turned out to be unique and highly vascular selective due to the almost exclusive expression of the VEGF receptors by vessel-forming endothelial cells. Interestingly, VEGF had been identified by Harold Dvorak in 1983 as a potent inducer of permeability [designated as vascular permeability factor (VPF); ref. 4], but this earlier work did not recognize the ability of VPF to induce angiogenesis.

Following the discovery of VEGF, it took 15 years to develop VEGF blocking drugs and to translate these into clinical application, first for metastatic colorectal cancer (5) and thereafter to other tumor entities. The clinical approval of the VEGF neutralizing antibody bevacizumab in oncology was a paradigm-changing breakthrough, because it was the first clinically effective tumor therapy that did not target the tumor cells, but the tumor-associated stroma. Yet, the road toward the clinical implementation of VEGF-targeting therapies was a bumpy one. In 1998, at the peak of angiogenesis research, the *New York Times* published an article in which it quoted Nobel Laureate James D. Watson as saying: "Judah [Folkman] is going to cure cancer in two years." The resulting hype was followed by sobering results in clinical trials revealing that angiogenesis inhibitors were not effective when given as monotherapy, which essentially disproved the earlier hypothesis of Folkman that angiogenesis inhibition should keep tumors in an avascular state of dormancy. Instead, subsequent combination trials showed that an angiogenesis inhibitor, when given in combination with chemotherapy, was effective in significantly prolonging progression-free survival and overall survival (OS) of patients with cancer. For patients with colorectal cancer, this translated into a 25% extension of OS (from 15.6 to 20.4 months; ref. 5). Because the clinical approval of bevacizumab, first for patients with colorectal

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Cancer Res 2022;82:15–7

doi: 10.1158/0008-5472.CAN-21-3515

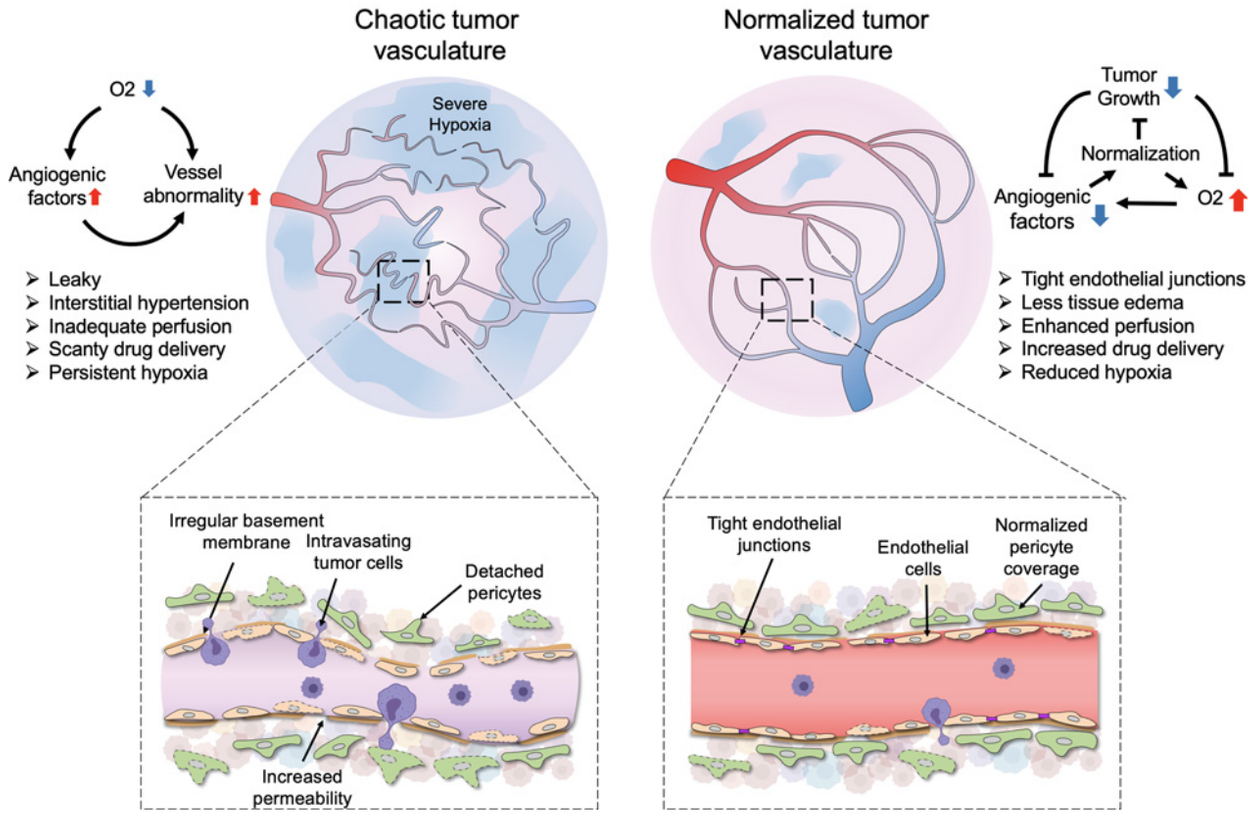

Figure 1.

Schematic presentation of tumor vessel normalization. Left, the chaotic tumor vasculature with leaky blood vessels results in high interstitial pressure, hypoxia, and inadequate perfusion, which leads to poor drug delivery. Right, antiangiogenic tumor therapy prunes the immature tumor vasculature resulting in a more normal appearing and perfused vasculature. Antiangiogenesis thereby facilitates better access of chemotherapy.

cancer and later for other tumor types, more than 15 angiogenesis inhibitors were approved for use in various indications in oncology and ophthalmology. In terms of market share, two antiangiogenic drugs have made it into the list of the top 10 selling drugs globally.

The successful clinical implementation of antiangiogenic therapies was a major milestone in oncology. Nevertheless, it created a scientific dilemma, because it is not readily understood why antiangiogenesis would not work as monotherapy, but only in combination with chemotherapy. In fact, the opposite would be expected. If angiogenesis inhibition would halt the growth of blood vessels and lead to the regression of the tumor-associated vasculature, then chemotherapy should not get better, but worse access to the tumor. This conundrum was solved by Rakesh K. Jain's group in Massachusetts General Hospital at Harvard Medical School (Boston, MA). Tong and colleagues showed in a landmark 2004 study published in *Cancer Research* that angiogenesis inhibition did not lead to global regression of tumor-associated blood vessels. Instead, it caused the selective "pruning of immature vessels and improvement of the integrity and function of the remaining vasculature by enhancing the perivascular cell and basement membrane coverage" (6). The authors called this "vascular normalization" because the chaotic tumor vasculature appeared and functioned more normally (**Fig. 1**). As a result, antiangiogenesis strategies would not starve a tumor to death, but instead facilitate better access of chemotherapy through normalized tumor perfusion. In fact, Jain and coworkers moved on to show in another *Cancer Research* publication that increased survival of patients with glioblastoma responding to antiangiogenic therapy was associated with elevated blood perfusion (7). Mechanistically, these findings are the opposite of Folkman's earlier prediction on how antiangiogenic tumor therapy would work.

The concepts of vascular normalization are nowadays well established. A recent PubMed search identified more than 600 papers with the search string "vessel/vascular normalization" published since the first description of this phenomenon in *Cancer Research*. Yet, what is not at all known to this day is what the relative contributions of vessel regression (i.e., starving the tumor) versus vessel normalization (i.e., facilitating chemotherapy) are toward the clinical efficacy of antiangiogenic tumor therapy. The answer to these and other burning questions are urgently needed to rationally advance antiangiogenic tumor therapy in a knowledge-based mechanism-driven manner.

Antiangiogenic tumor therapy today is a double-edged sword. On the one hand, it has become part of standard tumor therapy. However, it has not lived up to the high expectations it had in the early days of translational angiogenesis research. In fact, one cannot refrain from concluding that the full potential of antiangiogenic tumor therapies has probably not yet been exploited. General wisdom has taught that "targeted therapies require targeted diagnostic procedures." In simple words, one would not give a Her2 receptor blocker to a patient with Her2-negative breast cancer. As for angiogenesis inhibitors, there are to this day no established stratifying diagnostic procedures that would

predict if a patient with cancer would benefit from antiangiogenic therapy or not. As a result, angiogenesis inhibitors are often given to the wrong patients. Clearly, the poor understanding of the specific properties of the human tumor vasculature is a major bottleneck in further rationally advancing antiangiogenic tumor therapies because, realistically speaking, it is today less clear than ever what the main objective of antiangiogenic intervention is, namely vascular regression or vascular normalization (or both).

Work in preclinical mouse models has paved the way toward human translation. While the mechanisms of angiogenesis in mice and humans are essentially identical, the spatiotemporal dynamics and kinetics of mouse and human tumor growth are very different. This may be the most important reason why preclinical therapy studies in mice can oftentimes not readily be translated into humans. Vascular regression works very effectively in rapidly growing mouse tumors. Yet, the fine-tuned balance of therapy-induced vascular regression versus vessel normalization is more difficult to mimic in mouse tumor models. Thus, a better understanding of the specific properties of the human tumor vasculature is needed to implement therapy-stratifying diagnostic procedures, particularly because the next wave of antiangiogenic combination therapies has already entered the clinic. Immunotherapies with immune checkpoint inhibitors have dramatically changed tumor therapy in the last decade, because therapeutic manipulation of the endogenous immune system has the potential to be curative for tumor patients. While the prospects of immunotherapies are enormous, their limitation to this day is that they work effectively only in smaller subpopulations of patients with tumor. Thus, the holy grail to advancing immunotherapies at the moment is to implement combination therapies that improve the efficacy of immune checkpoint inhibitor therapy. Antiangiogenesis may be part of the solution to these enigmatic questions: Elegant preclinical work has shown that antiangiogenesis has the potential to substantially improve the efficacy of immunotherapy (8, 9). Intriguingly, these spectacular preclinical studies have in part already been translated into the clinic. Recent clinical trials in hepatocellular carcinoma have shown that the addition of antiangiogenic therapy to immunotherapy dramatically extends OS compared with the established standard of care (10). These recent findings may be considered the most important breakthrough in translational angiogenesis research since the clinical approval of the first angiogenesis inhibitor in 2004. Yet, they also stimulate many new burning questions that await to be answered to rationally advance combination therapies in a mechanism-based manner. Notably, is vascular normalization at the heart of better facilitating access of T cells into tumors or are antiangiogenic drugs also acting as immunomodulators beyond their effects on blood vessels?

Angiogenesis research has come a long way in the last 50 years. The concepts of "vessel normalization" pioneered in a *Cancer Research* publication in 2004 (6) have fundamentally changed the approach to implementing antiangiogenic tumor therapies. Building on the pioneering work of Tong and colleagues, a strongly intensified research effort is urgently needed today at the interface of preclinical model-based research and analytic clinical and pathology-based studies to better understand the nature of the tumor vasculature in human tumors. This will be a prerequisite to further advance antiangiogenic therapy in the clinic, which are today probably more appropriately conceptualized as "vascular stroma reprogramming therapies."

Authors' Disclosures

No disclosures were reported.

Received October 16, 2021; accepted November 3, 2021; published first January 1, 2022.

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Cancer Res 2022;82:15-17.