

Tumor Cell Populations Differ in Angiogenic Activity: A Model System for Spontaneous Angiogenic Switch Can Tell Us Why

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Aggressive, life-threatening cancers grow progressively locally, produce distant metastases, and are insensitive to cytotoxic and cytostatic drugs. A hallmark of progressive cancer growth is the subversion of the vascular supply in the whole area that is involved with tumor. In 1945, Glen Algire published in this Journal studies supporting the conclusion that “the rapid growth of tumor explants is dependent on the development of a rich vascular supply” (1). We now know that many tumors are dependent on neovascularization to sustain their expansion, that tumor cells themselves can produce proangiogenic factors, and that certain drugs targeting these factors can be effective anticancer drugs.

Tumorigenesis represents a multistep process that leads to the accumulation of genetic alterations, including gain-of-function mutations in oncogenes and loss-of-function mutations in tumor suppressor genes (2). One such step is known as the “angiogenic switch,” via which tumor cells acquire the ability to induce sprouting of new vessels from the surrounding quiescent vasculature (3,4). In some cases, the angiogenic switch has been linked to mutations of oncogenes or tumor suppressor genes. For example, the p53 tumor suppressor protein regulates the antiangiogenic factor thrombospondin 1, such that loss of p53 function, which is common to many tumor cells, reduces the levels of thrombospondin 1 (5). In other examples, both activation of the ras oncogene and loss of the von Hippel–Lindau tumor suppressor gene promote the expression of vascular endothelial growth factor (VEGF), resulting in increased endothelial cell growth and angiogenesis (6,7). Unlike normal postnatal angiogenesis, which is observed physiologically in the ovary and the endometrium or as part of wound healing in injured tissues—in which the new vessels look normal and the process is self-limited and carefully regulated—in cancer, the new vessels are often structurally and functionally abnormal and the process does not stop, due in part to the defective nature of the tumor vasculature, which is tortuous and leaky, leading to tissue hypoxia and further stimulation of angiogenesis (8).

Autopsy studies have led to estimates that almost all adults between the ages of 50 and 70 years have small thyroid carcinomas, only 0.1% of which are clinically apparent, and that 39% of women between the ages of 40 and 50 years have foci of breast cancer, only 1% of which are clinically apparent (9). All of us may thus harbor some cancer cells in our tissues, particularly later in life, and our fate may depend on whether such cells turn into an aggressive cancer. It is important to know the steps required for such small cancer foci to grow into lethal cancers and how to prevent the process. In this issue of the Journal, Naumov et al. (10) have focused on the angiogenic switch that is observed when small dormant cancers change into cancers that grow fast and progress. They started from established breast adenocarcinoma, osteosarcoma, and glioblastoma cell lines, which they injected into severe combined immunodeficient (SCID) mice. Tumors either did not form or remained microscopic and harmless for more than 100 days, sometimes as long as a year, but eventually a proportion of tumors started to grow progressively. The authors termed these

growing tumors “angiogenic” because they appeared red and were vascularized, as opposed to the dormant cancers, which they termed “nonangiogenic” because they appeared white and poorly vascularized. Cells derived from the angiogenic tumors were re-injected into SCID mice. This time, the tumors grew much more rapidly in the mice and became palpable by 21 days after injection. Thus, by using this method, the authors had selected from three established cell lines subpopulations of tumor cells with markedly different characteristics with respect to tumor formation in mice—that is cells forming indolent tumors, at least indolent for quite some time, or cells forming rapidly growing tumors. The authors looked for differences that could explain these divergent phenotypes. Both cell types had similar proliferation rates in vitro, but compared with nonangiogenic tumors, angiogenic tumors produced substantially greater amounts of the proangiogenic factor basic fibroblast growth factor (bFGF, also referred to as fibroblast growth factor 2, or FGF2) and substantially lower levels of the antiangiogenic factor thrombospondin 1. Levels of VEGF were similarly elevated in nonangiogenic and angiogenic breast carcinoma and glioblastoma cell lines, but angiogenic osteosarcoma cells produced substantially more VEGF than nonangiogenic osteosarcoma cells. Thus, in these examples, the ability of tumor cells to generate indolent or rapidly growing tumors correlated directly with levels of secreted proangiogenic bFGF and indirectly with levels of antiangiogenic thrombospondin 1.

Many activator and inhibitor proteins orchestrate angiogenesis. In addition to the FGF and VEGF protein families, other proangiogenic factors include platelet-derived growth factors, angiopoietins, placental-like growth factor, hepatocyte growth factor, transforming growth factors, stromal-derived factor 1, interleukin 8, and monocyte chemoattractant protein 1 (11). In addition to thrombospondin 1, negative regulators of angiogenesis include the Notch ligands Delta4 and Jagged1 (12,13), vasohibin (14), endostatin (15), angiostatin (16), vasostatin (17), and tumstatin (18). Blood vessels in different tissues may differ phenotypically (19), and at least one tissue-specific proangiogenic factor has been identified (20), suggesting the possibility of tissue-specific regulation of angiogenesis, including the angiogenic switch. Recent studies have explored the possible role in angiogenesis of axon guidance receptors and their ligands, including semaphorins and plexin receptors, Slits and Robo receptors, ephrins and Eph receptors, and netrins-DCC/neogenin and Unc5 receptors (21–23). In some cases, the same factors that provide axons with attractive and repulsive cues have been found to regulate migration and

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sprouting in endothelial cells. For example, EphB4 can attract endothelial cells and promote angiogenesis, including tumor angiogenesis, through ephrinB2 reverse signaling (24). Tumors expressing Sema3F can repel endothelial cells *in vivo* and display reduced vascularity; they are encapsulated and nonmetastatic (25). It will be interesting to extend the observations by Naumov et al. to broadly examine expression of known regulators of angiogenesis in nonangiogenic and angiogenic tumor cell subsets.

Recent studies have proposed the existence of tumor stem cells, *i.e.*, rare tumor cells within the bulk of proliferating tumor mass with self-renewing potential, quiescence of cell cycling, relative resistance to growth factors, and an ability to differentiate into diverse tumor components (26). Such cells would play a critical role in sustaining tumor growth (27). An important question is whether tumor stem cells are proangiogenic. Naumov et al. derived a tumor clone, A1, from an angiogenic breast tumor. Although it was derived from a rapidly growing tumor, this clone formed tumors in only 20% of injected mice and only following a prolonged dormancy of about 1 year. Thus, the A1 clone displayed some of the characteristics—the ability to self-renew, maintain relative quiescence and differentiate—of tumor stem cells. It is noteworthy that the dormant tumors from the A1 clone were nonangiogenic *in vivo* for a long time, given that other studies have suggested that the stem cell populations within breast cancer may promote angiogenesis more vigorously than the non-stem-cell bulk of the tumor cells (28). By depriving tumor stem cells of the necessary nutrients, antiangiogenic therapies could be particularly effective against these cells, which might otherwise be relatively resistant to cytotoxic chemotherapy.

It is clear that the acquisition of an angiogenic phenotype is one of the rate-limiting steps for sustained growth of certain tumors but is not itself sufficient for manifestation of a locally invasive and metastatic phenotype. Several highly vascular tumors, such as myomas uteri and angiomas, can grow to a relatively large size but do not usually become life-threatening. Also, a proportion of cancers may not require an angiogenic switch and may rather derive sufficient nutrients by co-opting existing host vessels (29). Nonetheless, most tumor growth is crucially dependent on the formation of a neovascular supply.

In 1971, Judah Folkman proposed the idea of using inhibitors of angiogenesis to treat cancer (30). During the last 2 years, the U.S. Food and Drug Administration approved two such inhibitors. Bevacizumab (Avastin), a humanized monoclonal antibody against VEGF, was approved for the treatment of metastatic colorectal cancer in combination with chemotherapy, and sorafenib (Nexavar), a synthetic inhibitor of several serine/threonine and receptor tyrosine kinases, including VEGF receptor 2, was approved for the treatment of advanced renal cell carcinoma. Many other drugs that block angiogenesis are currently under development (11). Based on the observations made by Naumov et al. in this issue and by many others, particularly interesting approaches would be to prevent the angiogenic switch or to turn it off before tumors acquire a large blood supply. Understanding the molecular basis for the angiogenic switch and how to regulate it seems like a very good start.

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