What Is the Evidence That Tumors Are Angiogenesis Dependent?

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In this issue of the journal, Kreisle and associates (1) report that angiogenesis and growth of B16 melanoma decreased with age in C57BL/10 mice. They suggest that age-related reductions in tumor growth may be secondary to a diminished neovascular response by the host. In another mouse tumor system (SP1 fibrosarcoma), in which tumor growth did not decline with age, the overall neovascular reaction elicited by the tumor from the host was sufficient to support the growing tumor. However, the new vessels in the old mice arose mainly from peripheral nerves, whereas in younger mice, they arose from subcutaneous tissue.

The behavior of tumors in both systems is consistent with the concept that “tumor growth is angiogenesis dependent” (2). This hypothesis, which was first proposed in 1971, can be stated in its simplest terms: Once tumor “take” has occurred, every increase in tumor cell population must be preceded by an increase in new capillaries converging on the tumor (3). The hypothesis has generated many studies leading to sequencing and cloning of angiogenic molecules (4); discovery of angiostatic steroids (5) as well as other angiogenesis inhibitors (6, 7); and elucidation of angiogenic diseases (4). The role of angiogenesis is now being explored in disciplines as diverse as developmental biology, cardiology, wound healing, ophthalmology, and dermatology.

While the supporting evidence for this hypothesis has been accumulated over more than a decade, it is scattered in the literature of basic science and clinical research. Thus, the experimental basis for the hypothesis is not always clear to investigators in angiogenesis research. Consequently, I have assembled this evidence and examined the limitations of the hypothesis.

The hypothesis has certain qualifications. It does not apply to the early phase of solid tumor growth. In this “prevascular” phase, tumors are usually thin and cell population is limited; some early in situ carcinomas contain fewer than 10^6 cells. Tumor cells may grow in ascites without neovascularization even when the cells are angiogenic.

Furthermore, the capacity of tumor cells to induce angiogenesis does not always correlate with malignancy, and there is considerable confusion in the literature about this fact. Adrenal adenoma, for example, is a benign tumor that is highly angiogenic. The onset of angiogenic activity in tumor cells is an independent event that may be expressed at very different times during neoplasia. Some examples are illustrated in table 1. These examples are not intended to imply that angiogenic factors originate exclusively from tumor cells per se. Other mechanisms are also involved in the switch to the angiogenic state during tumorigenesis, e.g., recruitment of macrophages (15).

It is helpful to think of the onset of angiogenesis as permitting rapid expansion of a tumor population, if the tumor cells are capable of rapid proliferation. From this perspective, the absence of angiogenesis in the prevascular phase precludes expansion of the tumor population regardless of the proliferative capacity of tumor cells. Tumor cell populations may escape the requirements of neovascularization by growing as a thin sheet of cells, e.g., on the meninges or within nerve sheaths.

The onset of angiogenesis also contributes to metastasis. Neovascularization permits the shedding of cells from the primary tumor (16), and decreased angiogenesis is associated with a decreased rate of metastasis (17).

The hypothesis that tumor growth is angiogenesis dependent rests in part on the following evidence:

(a) The growth rate of tumors implanted in subcutaneous transparent chambers in mice is slow and linear before vascularization and rapid and nearly exponential after vascularization (18).

(b) Tumors grown in isolated perfused organs where blood vessels do not proliferate are limited to 1–2 mm^3 but expand rapidly to 1–2 cm^3 after vascularization on transplantation to mice (e.g., 19).

(c) Tumor growth in the avascular cornea proceeds slowly and at a linear rate but switches to exponential growth after vascularization (e.g., 20).

(d) Tumors suspended in the aqueous fluid of the anterior chamber of the eye remain viable, avascular, and limited in size (<1 mm^3). Once they are implanted on the iris vessels, however, they induce neovascularization and grow rapidly, reaching 16,000 times their original volume within 2 weeks (e.g., 21).

(e) Human retinoblastomas metastatic to the vitreous or the anterior chamber are similarly avascular, viable, and growth restricted.

(f) Within a solid tumor, the [^3H]thymidine labeling index of tumor cells decreases with increasing distance from the nearest open capillary. The mean labeling index for a given tumor is a function of the labeling index of the vascular endothelial cells in that tumor (e.g., 22).

(g) Tumors implanted on the chorioallantoic membrane of the chick embryo are often restricted in growth during the avascular phase (>72 hr), but rapid growth begins within 24 hours after vascularization. In one study, tumors did not exceed a mean diameter of 0.93 ± 0.29 (SD) mm during the avascular phase, but after vascularization, tumors reached a mean diameter of 8.0 ± 2.5 mm by day 7 (23) (fig. 1).

(h) The chorioallantoic membrane appears on day 5 in
Table 1. Examples of time of onset of angiogenesis in different tumors

<table>
<thead>
<tr>
<th>Time of onset of angiogenesis</th>
<th>Animal</th>
<th>Human</th>
</tr>
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<tbody>
<tr>
<td>Before neoplasia</td>
<td>Mouse sarcoma (8)</td>
<td>Cervical cancer (9)</td>
</tr>
<tr>
<td>Coincident with neoplasia</td>
<td>Mouse pancreatic cancer (10)</td>
<td>Bladder cancer (11)</td>
</tr>
<tr>
<td>After neoplasia</td>
<td>Mouse fibrosarcoma (13)</td>
<td>Melanoma (14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ovarian cancer (peritoneal implants)</td>
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The data assembled here and the report by Kreisle and colleagues (1) provide mainly correlative and indirect evidence for the role of angiogenesis in tumor growth. However, recent experiments have begun to yield direct evidence. For example, Gross and co-workers (28) have reported a human colon carcinoma that lacks high-affinity receptors for basic fibroblast growth factor (bFGF) and for which bFGF is not mitogenic in vitro. When the tumor was grown in nude mice, systemic administration of bFGF intraperitoneally stimulated increased blood vessel density and branching in the tumor as well as a twofold increase in tumor size. Administration of neutralizing monoclonal antisera to bFGF significantly retarded tumor growth. Receptor autoradiography of histological tumor sections demonstrated that receptors for bFGF were on the vascular endothelium.

It may eventually be possible to demonstrate causality in other ways, e.g., by activating a cancer-suppressor gene that regulates an angiogenesis inhibitor (7) or by modifying the rate of switching to the angiogenic state in hyperplastic islets of the pancreas in transgenic mice (10). Meanwhile, continued progress in the development of angiogenesis inhibitors toward the goal of future tumor therapy is a fruitful outcome of the concept that tumors are angiogenesis dependent.

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**Figure 1.** Growth curves of Walker 256 rat carcinoma implanted on chick chorioallantoic membrane from day 5 to day 13 (24). Arrows indicate beginning of vascularization, which occurs at ~72 hrs (23). These data demonstrate different growth rates of tumor in prevascular and vascular phases. Slowing of tumor growth begins before immunocompetence in embryo. Values = mean ± SD.
References

(14) SRIVASTAVA A, LAIDLER P, DAVIES R, ET AL: The proangiogenic activity in intermediate-thickness (0.76-4.0 mm thick) skin melanoma. Am J Pathol 133:419-423, 1988

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Vol. 82, No. 1, January 3, 1990