Commentary

Can mosaic tumor vessels facilitate molecular diagnosis of cancer?

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The hypothesis that tumor growth is angiogenesis-dependent (1) has been supported by extensive experimental evidence (for reviews see refs. 2 and 3), and has been confirmed by genetic methods (3–5). The switch to the angiogenic phenotype is a critical point in tumor progression (6). Before this switch occurs, most human and animal tumors are restricted to a microscopic size. An example is early *in situ* carcinoma where neighboring microvessels are quiescent and mature, and metastases are virtually nonexistent. After the angiogenic switch, for example in later stages of *in situ* breast carcinoma, neovascular sprouts breach the basement membrane (7), and tumor cells can grow around each new capillary vessel (Fig. 1 *A* and *B*), enter the circulation, and form metastases. One endothelial cell can support more than 50 to 100 tumor cells. Thus, the microvascular endothelial cell recruited by a tumor has become an important second target in cancer therapy. Treating both the cancer cell and the endothelial cell in a tumor may be more effective than treating the cancer cell alone (2, 8). As a result, angiogenesis inhibitors have emerged as a new class of drugs. These drugs selectively or specifically inhibit proliferation or migration of activated endothelial cells, or induce their apoptosis. Tumor growth is inhibited or tumors regress. Angiogenesis inhibitors are currently being tested in clinical trials alone, as well as in combination with chemotherapy or radiotherapy.

The paper by Chang *et al.* (9) employs novel elegant methods to quantify traffic of tumor cells traversing new microvessels (Fig. 1*B*). Researchers from two leading laboratories of vascular biology demonstrate that tumor cells in transit to the vascular lumen may reside temporarily in the microvessel wall and occupy up to 4% of the total vascular surface area. Approximately 15% of vessels in a human colon carcinoma in mice contain a subpopulation of tumor cells that share space in the vessel wall with endothelial cells. These are called "mosaic" vessels. The authors calculate that half of the tumor cells exposed to the vessel lumen are shed into the circulation in a given day, and they note that this rate of tumor cell intravasation would be consistent with previous reports that up to one million cells are shed per gram of tumor per day.

These findings have fundamental implications for cancer biology and for cancer therapy. For example, endothelial cells stimulated by mitogens such as basic fibroblast growth factor (bFGF) or vascular endothelial cell growth factor (VEGF), secrete metalloproteinase-2 (gelatinase A) which contributes to degradation of basement membrane in microvessel walls (10). This breakdown in the vascular basement membrane may facilitate extravasation of endothelial cells during the formation of neovascular sprouts (Fig. 1*C*), as well as intravasation of tumor cells into the lumen (Fig. 1*B*). The angiogenesis inhibitor endostatin is a 20-kDa fragment of collagen XVIII (11) currently in clinical trial for patients with advanced metastatic cancer.

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One of its functions is to inhibit endothelial cell and tumor cell invasion by blocking the catalytic activities of both metalloproteinase-2 and membrane type-1 metalloproteinase (12).

In addition to the relatively small subpopulation of tumor cells in transit across the microvessel wall, a third subpopulation of cells, progenitor endothelial cells circulating from bone marrow, may also enter the wall of new microvessels (13–15). Under the usual conditions of tumor angiogenesis, when endothelial cells in the tumor bed have been predominately recruited from the local neighborhood, only a minuscule fraction of these endothelial cells, if any, are derived from bone marrow progenitor endothelial cells (16). However, under experimental conditions when a tumor is unable to recruit endothelial cells from its local neighborhood, the bone marrow may be a major contributor of these cells. When one allele of the developmental gene Id1 and two alleles of Id3 are deleted, mice born with this defect are unable to mount a local angiogenic response to transplanted tumor cells, and the tumors either do not grow or grow slowly (4). In contrast, when these mice receive a transplant of wild-type bone marrow, progenitor endothelial cells marked by normal Id1 and Id3 arrive in the tumor bed in sufficient numbers to permit intense neovascularization of the tumor and rapid tumor growth (Robert Ben Ezra, unpublished data presented at the American Association for Cancer Research Symposium on Angiogenesis, Traverse City Michigan, October 2000). Taken together with the paper by Chang *et al.* (9), these results provide a dynamic picture of tumor neovasculature in which the endothelial cell lining itself is continuously migrating, whereas at the same time tumor cells in transit to the lumen are taking up temporary residence in the microvascular wall and a few progenitor endothelial cells may be arriving at the angiogenic site from the bone marrow. In contrast, little or no endothelial cell turnover or tumor cell traffic would be found within the walls of mature, quiescent microvessels covered with pericytes and contained by a stable nondegraded basement membrane. Angiogenesis inhibitors may generally be most effective in preventing growth of neovasculature or in causing regression of neovasculature, but have little or no effect on mature microvasculature.

Chang *et al.* (9) emphasize that "antivascular effects of some conventional anticancer therapies could be explained by mosaic vessels, because killing exposed cancer cells could impair blood flow in 14% of the vessels causing significant antivascular effects." This is an important point that would apply to most cytotoxic chemotherapeutic agents until they be-

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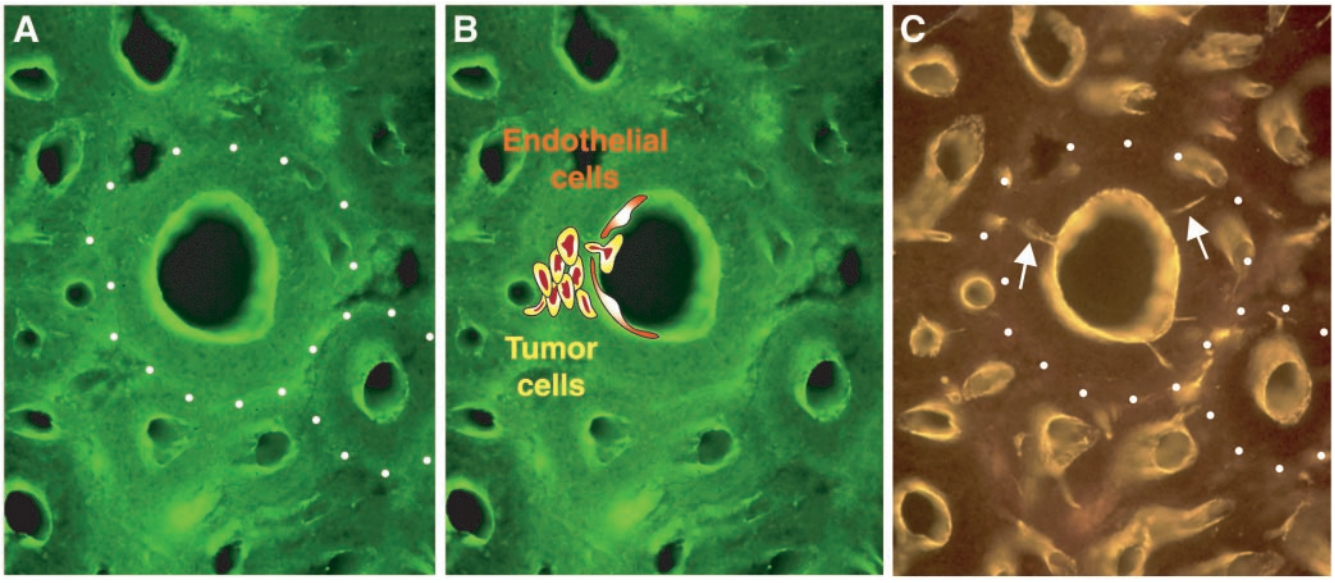

Fig. 1. Cross-sections of breast cancer (MCa-IV) in mice showing the microcylinders of tumor cells that surround each vessel. Large and small thin-walled microvessels in breast tumor labeled by vascular perfusion of green (FITC) fluorescent lectin staining (*A*), or by CD31 immunoreactivity viewed by Cy3 fluorescence (gold) (*C*). The perivascular cuff of tumor tissue, outlined by white dots in *A* and *C*, is 100 microns thick, which is within the range of the oxygen diffusion limit. In *B*, two endothelial cells (red cytoplasm with white nuclei) have been drawn facing the lumen to approximate scale. Yellow tumor cells with brown-red nuclei occupy the perivascular cuff of tumor tissue. One tumor cell is intravasating into the lumen and is exposed to the blood between the two endothelial cells. This tumor cell represents the approximately one million tumor cells per gram of tumor that may shed into the circulation each day. The CD31 immunoreactivity, like the lectin in *A* and *B*, defines the luminal surface of the vessels, but, unlike the lectin, it also labels tiny sprouts (white arrows), which have no apparent lumen because they have CD31 immunoreactivity, but no lectin staining. These sprouts of about 1 μm diameter radiate from the vessel lining into the 100 μm thick perivascular cuff of tumor tissue. The sprouts result from endothelial cells that are migrating (extravasating) from the wall of the microvessel. Vessels were preserved in the open state by vascular perfusion of fixative (courtesy of Donald M. McDonald, University of California, San Francisco) (2, 23). (Drawings in *B* by J.F. and Kristin Gullage.) [Reproduced with permission from ref. 2 (Copyright 2000, B. C. Decker).]

came drug-resistant. However, even tumors that had become "drug-resistant" to alkylating agents (i.e., cyclophosphamide), could still possibly kill tumor cells exposed in the vessel wall. Experimental tumors that were made drug-resistant to alkylating agents *in vivo* were not drug-resistant when grown in monolayers *in vitro*, but were highly drug-resistant when grown as multicellular *spheroids in vitro* (17, 18). Single tumor cells released from these spheroids almost immediately lost their drug resistance. This result suggested a possible mechanism of drug resistance in tumors treated with alkylating agents that was dependent on the response of a crowded cell population, "i.e., multicellular resistance as opposed to classic unicellular resistance mechanisms" (17). It remains to be demonstrated whether alkylating agents have, in addition to their direct cytotoxic effect on tumor cells, an antivascular activity that may escape acquired drug resistance because of microvascular mosaicism. However, it has been demonstrated that cyclophosphamide can be a potent direct angiogenesis inhibitor that causes apoptosis of endothelial cells, regression of neovasculature, and inhibition of tumor growth, even if the tumors are drug-resistant, as long as the cyclophosphamide is administered on an "antiangiogenic" low dose schedule instead of on a "conventional" maximum tolerated dose schedule (19).

Perhaps the most provocative implication of the paper by Chang *et al.* is that if tumor cell shedding into the circulation from approximately 15% of a tumor's vessels in mice also translates to humans, this would provide a further rational basis for the genetic analysis of circulating tumor cells (20, 21) isolated from blood, an effort currently underway in several laboratories. In the foreseeable future, such molecular methods could possibly diagnose cancer before it can be located by conventional imaging techniques. Without knowledge of a tumor's location, conventional surgery and radiotherapy are not useful, and conventional chemotherapy may be too harsh. If molecular diagnosis of cancer before a tumor can be located becomes common, there will be an increasing need for nonharsh therapies that can circumvent drug resistance. These include among others, antiangiogenic therapy, immunotherapy (including vaccine therapy), gene therapy, and "antiangiogenic" low-dose chemotherapy. If endothelial cells or their apoptotic bodies (22) are shed from a tumor bed into the circulation, it may be possible to determine that there is an angiogenic site in the body, either by analyzing these cells or their membrane proteins (e.g., ephrins) from a blood sample. These possibilities are of course only speculative, but they are just a few of the far-reaching implications of this important quantitative analysis of mosaic tumor vessels.

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