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Perspectives and Commentaries

What is the Role of Angiogenesis in Metastasis from Cutaneous Melanoma?

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(A COMMENT ON: Srivastava A, Laidler P, Hughes LE, Woodcock J, Shedden EJ. Neovascularization in human cutaneous melanoma: a quantitative morphological and Doppler ultrasound study. *Eur J Cancer Clin Oncol*, 1986, **22**, 1205–1209.)

BRESLOW first proposed in 1970 that a cutaneous melanoma with a thickness of less than 0.76 mm was associated with low metastatic rate and high curability [1]. The paper raised two questions in the minds of oncologists: (i) Would such a sharp breakpoint continue to hold up, or would its range and standard deviation gradually increase as subsequent authors reported their experiences? (ii) Why should a minute change of tumor thickness be responsible for such a sharp delineation between low and high metastatic rate?

The first question generated clinical studies from melanoma centers throughout the world. More than a decade-and-a-half later, it is clear that Breslow's prediction has stood the test of time. Tumor thickness remains the single most important indicator of prognosis in patients with cutaneous melanoma of the nodular or superficial spreading type [2]. Also, a thickness of 0.76 mm or less continues to correlate with the lowest metastatic rate, occasional proposals to modify this measurement notwithstanding [3].

The second question remained an enigma until the mid-1970's when experiments by Folkman and his associates demonstrated that tumors begin their growth in an avascular (or prevascular) phase followed by a vascular phase in which further tumor growth is angiogenesis-dependent [4]. In the avascular phase, growth is restricted because nutrients and catabolites can be exchanged only by simple diffusion with the existing capillary bed. In contrast, once a tumor is vascularized by new capillaries, rapid tumor growth is possible. On the basis of these studies, it was proposed that most carcinomas probably originate in the avascular epithelial compartment as *in situ* lesions, and remain separated from the vascular bed until the basement membrane is breached, either by the tumor itself, or by new capillary vessels, or by both [4].

Now, in an important paper, Srivastava *et al.* [5] report that in patients with cutaneous melanomas more than 0.9 mm thick, there is evidence of tumor neovascularization as demonstrated by Doppler detection of blood flow, and by histological quantitation of small blood vessels. In most melanomas less than "0.75 mm thickness", tumor neovascularization appears to be absent. This may be the first report of a correlation between thickness of cutaneous melanoma and tumor vascularity. Furthermore, there is a positive correlation between velocity of blood flow and vessel density and vascular area.
and vascular area.

The increased Doppler signals may be the result not only of an increased number of new vessels, but also an increased diameter of the new vessels as reported by these authors. Among the new vessels induced by tumor, it is common to see abnormally large capillaries with 3, 4, or more endothelial cells per lumen; smooth muscle cells are lacking. In contrast, a cross-section of normal skin capillaries reveals 1 or 2 endothelial cells; vessels with more endothelial cells are wrapped in smooth muscle. It is likely that large capillaries at the tumor base, as reported by Srivastava *et al.* [5] arise from rapid

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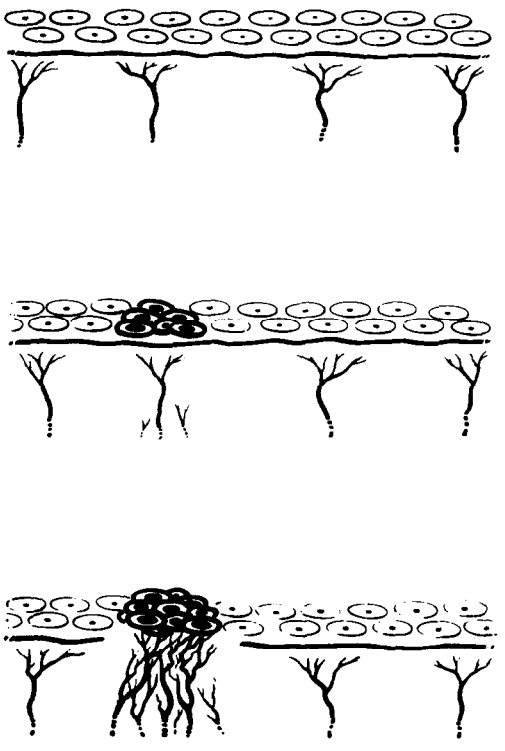

Fig. 1. Diagram of theoretical progression of a carcinoma which arises in an avascular epithelial compartment separated from the vascular bed by basement membrane. Conversion to the vascularized phase requires degradation of the basement membrane. The tumor could be melanoma, carcinoma of the cervix in situ, *or a malignancy arising in any other epithelial lining. (From Folkman and Cotran* [4], *with permission of the publisher.)*

proliferation of capillary endothelial cells.

Why does an increased tumor thickness beyond a critical point correlate with the onset of neovascularization? Most experimental data would indicate that a cutaneous melanoma must induce new capillary growth *before* it can increase its thickness; i.e. tumor growth beyond a critical thickness is angiogenesis-dependent [6]. It is thus possible that 0.76 mm is approximately the longest distance permissible between the outer layer of melanoma cells and the nearest open capillaries. The experimental evidence for angiogenesis-dependence of tumor growth turns on the severe restriction of tumor size imposed by lack of neovascularization. For example, if tumors are prevented from becoming vascularized in the anterior chamber of the rabbit eye, they do not grow beyond about 0.6 mm^3 [7]. However, if allowed to become vascularized they reach a volume of approximately 330 mm^3 in the same period of time. Other evidence is based on the following observations: that the ^{3}H-thymidine labelling index of tumor cells decreases with increasing distance from an open capillary [8, 9]; that as normal cells progress to neoplasia, angiogenic capacity appears before the cells become tumorigenic [10]; and that the administration of angiogenesis inhibitors can suppress tumor growth in some tumor-bearing animals [11].

It should be remembered, however, that the dependency of solid tumors upon angiogenesis is related to growth of their cells in a tightly packed population of high density (10^8–10^9 cells/cm^3). For example, tumor "take" does not require angiogenesis because microscopic-sized tumor populations can survive near existing capillaries. Also, angiogenesis may not be required by malignant cells which have developed the capacity to grow at lower density and separated from each other (e.g. ascites). Certain tumor configurations may also permit freedom from angiogenesis. On the basis of experimental evidence, radial growth of a cutaneous melanoma could continue in the absence of neovascularization, whereas vertical growth beyond a critical thickness would require neovascularization.

Why is a "thin" melanoma (i.e. 0.76 mm or less) associated with a relatively low risk of metastasis? There are many sequential steps in the metastatic process, but entry of tumor cells into the circulation is a pre-requisite for distant metastases. Experimental studies indicate that metastatic potential is abrogated or markedly reduced in the absence of neovascularization. Shedding of tumor cells into the circulation is not observed until after tumors have become vascularized [12]. Once vascularization has occurred, the number of cells which enter the microvasculature is linearly related to the tumor volume and the total surface area of tumor vessels [13]. Entry of tumor cells into tumor vessels may be facilitated by the angiogenic stimulus itself. *In vitro* studies show that tumor-derived angiogenic factors induce capillary endothelial cells to release enzymes which can locally degrade basement membrane [14], and that tumor cells can envelope new capillary sprouts and prefer to grow in contiguity to these sprouts, even in the absence of flow [15].

If the work of Srivastava *et al.* [5] holds up in larger series of melanomas, the new insights which it provides may be generalized to a variety of carcinomas. Is there a "thin" prevascular stage of carcinoma of the breast, colon, or lung, which we cannot now detect by conventional methods? Could such a lesion behave in a manner analgous to melanoma and remain in this state possibly for years before converting to the vascularized phase? The biology of carcinoma of the cervix *in situ* and of certain carcinomas of the bladder suggests that this might be the case. Will it be possible some day to know when angiogenic activity has turned on in any part of the body? This research goal seems worth pursuing, especially since tumor-derived angiogenic factors have recently been completely purified [16, 17]. The report of Srivastava *et al.* affords the opportunity to conceptualize such an objective.

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