Angiotropism in Cutaneous Melanoma: A Prognostic Factor Strongly Predicting Risk for Metastasis

To the Editor:

The role of tumor vascularity in the neoplastic progression and potentially the prognosis of cutaneous melanoma is of singular importance (Barnhill *et al*, 1996). Both angiogenesis and vascular/lymphatic invasion have been the focus of considerable study as biologic phenomena and potential prognostic factors (Barnhill *et al*, 1996; Busam *et al*, 1996). Despite a number of studies on microvessel density, as a correlate of angiogenesis, there are no definitive data that such microvessel counts constitute a prognostic factor in melanoma (Barnhill *et al*, 1996). Vascular and/or lymphatic invasion, i.e., the presence of tumor aggregates within the latter channels, has been thought to be a direct manifestation of metastasis in progress and accordingly a prognostic factor (Busam *et al*, 1996). In fact, the observation of vascular/lymphatic invasion in tissue sections is exceedingly rare and often an artifact that can be attributed to tortuous vascular channels folding back into the vascular lumina and also tissue shrinkage resulting in the appearance of a (false) vascular space. Thus at least in some instances the tumor aggregates are in fact external to the vascular/lymphatic channel rather than being intraluminal. As result we have questioned the utility of microvessel quantification and the practical value of observing vascular invasion as prognostic factors in cutaneous melanoma.

Because of the observations mentioned above and our previous studies demonstrating a unique association between melanoma cells and the abluminal surfaces of endothelial cells, we have focused on this relationship between tumor (melanoma) cells and the external surfaces of vessels both as a mechanism of tumor metastasis and as a potential prognostic factor. Indeed we have identified a hitherto unrecognized image – the angio-tumoral complex – in which the tumor cells and endothelium are in contact via an amorphous matrix containing specific laminin(s), including potentially the β2 chain of laminin (Lugassy *et al*, 1997; 1999a, b). In the angio-tumoral complex, endothelial cells show no sign of physiologic damage, no tumoral intravasation, and tumor cells occupy a pericytic location. Given the role of laminin in migration, metastasis, and protease activity, we have suggested that this periendothelial free-laminin is promoting the migration of melanoma cells over the mesenchymal surface of the endothelium. This potential mechanism of tumor spread has been termed "extravascular migratory metastasis", as opposed to intravascular tumor spread (Lugassy *et al*, 1999b).

Angiotropism (melanoma cells cuffing the external surface of vessels) is likely to be the histopathologic counterpart of the angio-tumoral complex. We have noted angiotropism to be a much more frequent pathologic finding than that of vascular or lymphatic invasion. Thus angiotropism (and the angio-tumoral complex) may not only potentially represent a mechanism of melanoma spread along the external surface of endothelium via specific laminin(s), but also a prognostic factor of metastasis.

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We have studied the prognostic significance of angiotropism as a qualitative parameter, i.e., one that is recorded as present or absent *versus* a continuous variable such as numbers of microvessels that lends itself to quantification, in a series of patients with primary cutaneous melanoma and documented metastasis matched with a similar group of patients with nonmetastasizing primary melanomas. The cases were retrieved from the following institutions: the Massachusetts General Hospital, Brigham and Women's Hospital, Johns Hopkins Hospital, George Washington University Medical Center, and the institutions cooperating with the Cancer Prevention Research Unit at Yale University. Eighty patients with one or two representative slides and definite outcomes or follow-up were enrolled in the study. The 80 patients comprised two groups: (i) 40 patients with melanomas metastasizing to regional lymph nodes, visceral sites, or both, and (ii) 40 patients with nonmetastasizing melanomas and long-term disease-free follow-up (range 5–22 y, mean 10.77 y). Another component of the study involved the matching of patients from the two groups above for the following major prognostic factors: tumor thickness in millimeters (usually allowing no more than a 20% difference); age in years (usually no more than a decade difference); gender, i.e., males were matched with males and females with females; and anatomic site matched for three major regions – head and neck, trunk, and extremities. Twenty-six pairs of patients resulted from this matching. In general only one or two slide(s) from each melanoma were available for review, and this slide or slides was comprehensively assessed by a single observer (RLB) for angiotropism and vascular/lymphatic invasion without knowledge of patient outcome. All tissue sections on each microslide or slides were systematically studied microscopically for evidence of angiotropism (as defined below) at the advancing front of the melanoma and up to 1–2 mm beyond the main tumor mass. Among the two groups of patients with and without metastases, each comprised of 40 melanomas, 99 and 71 tissue sections, respectively, were reviewed from each group. For the vast majority of cases in both groups only one or two tissue sections were available for review. Among the cases with definite angiotropism five or six tissue sections were available for each of five cases, whereas among 12 cases without angiotropism four to eight tissue sections per case were available for study. Thus number of sections available for review did not influence significantly the likelihood of detecting angiotropism. Serial step sections were not performed as the tissue block was not available for almost all of the tumors.

Angiotropism was defined as multiple melanoma cells singly disposed along or in aggregates closely opposed to the external surfaces of (and not within) microvessels and/or lymphatics at the advancing front and/or some distance (1–2 mm) away from the main part of the tumor (**Fig 1**). Angiotropism was graded as (i) absent (absence of tumor cells clearly cuffing vessels), (ii) equivocal (a single focus of tumor cells clearly cuffing a vessel), or (iii) definitely present (two or more foci of tumor cells clearly cuffing a vessel). Angiotropic melanoma cells were recognized by their unequivocal similarity to nearby melanoma cells; if there was any question about the identity of the cells cuffing vessels the case was scored as negative for angiotropism. Five cases with definite angiotropism underwent immunostaining with polyclonal antibodies directed against S 100 protein and CD 31, a vascular marker,

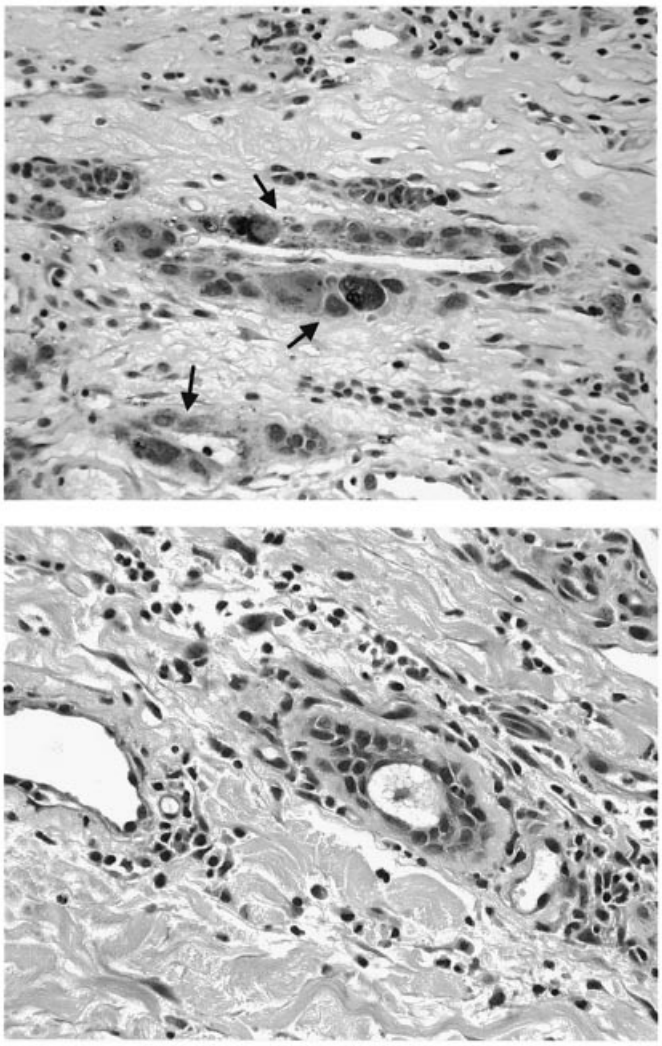

Figure 1. (*a*) Primary malignant melanoma, invasive, with measured depth of 3.2 mm and demonstrating angiotropism, and melanoma cells identified by immunostaining with S 100 protein cuffing two microvessels (arrows). (*b*) Cutaneous melanoma with angiotropism. *(Scale bar = 50mm).*

to determine if such immunostaining of angiotropic melanoma would aid in the recognition of angiotropism.

Among the 40 pairs of patients, there were no significant differences with respect to age (mean 50.7 y for patients with metastases *versus* 53.0 y for patients without metastases, p = 0.543, Student's two-tailed *t* test); however, patients with metastasizing melanomas had thicker melanomas than those without metastases (2.2 mm *vs* 1.6 mm, p = 0.016). The 40 patients with metastasizing melanomas showed definite angiotropism (**Fig 1*a***) in 16 cases and equivocal angiotropism in five cases *versus* no definite angiotropism in any case and equivocal angiotropism in six cases among the 40 melanomas without metastasis (p = 0.00005, Fisher's exact test). Melanomas with angiotropism had a mean thickness of 2.3 mm and thus were significantly thicker than melanomas without metastases (p = 0.0058). We found vascular/lymphatic invasion in only a single patient who had metastatic melanoma and also exhibited angiotropism. When the 26 matched pairs of patients were examined, there were no differences in age and tumor thickness (**Table I**). Seven patients with metastatic melanoma demonstrated definite and three equivocal angiotropism, however, *versus* no definite angiotropism and only two melanomas with equivocal angiotropism in the group without metastases (p = 0.008). Although there were too few cases for a definitive analysis, the presence or absence of angiotropism did not appear to have any effect on survival among the patients with metastases. Any potential effect of angiotropism on survival, however, requires further study with a larger number of patients and longer follow-up.

Table I. Analysis of 26 patients with primary melanoma associated with metastases matched with 26 patients without metastases

Characteristic	Metastases	Non-metastases	p-value
Age	55.8 y[a]	52.1 y	0.383[b]
Tumor thickness	2.1 mm[a]	1.9 mm	0.341
Angiotropism			
Absent	13	24	
Equivocal	3	2	
Present	7	0	0.008[c]

[a]Mean.
[b]Student's two-tailed *t* test.
[c]Fisher's exact test.

Immunostaining of the five cases with S 100 protein enhanced the recognition of angiotropic melanoma cells (**Fig 1*b***). The immunostaining with S 100 protein was not more sensitive than conventional microscopy but instead simply validated the observation of angiotropism. There was no significant benefit from the use of CD 31.

These findings demonstrate that angiotropism as defined here is more commonly observed histopathologically than vascular/lymphatic invasion in melanoma. A major outcome from this study was to show that angiotropism can be easily recognized by light microscopy in routine tissue sections of melanoma. The use of S 100 protein may facilitate the recognition or confirmation of angiotropism, particularly for those not experienced with this phenomenon. Furthermore our results strongly suggest that angiotropism is an important prognostic factor correlating with metastasis. Based on these qualitative results, we are in the process of carrying out more quantitative studies supplemented by immunohistochemistry with S 100 protein to confirm these observations.

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REFERENCES

Barnhill RL, Fine J, Roush GC, Berwick M: Predicting five-year outcome from cutaneous melanoma in a population-based study. *Cancer* 78:427–432, 1996

Busam KJ, Berwick M, Blessing K, *et al:* Tumor vascularity is not a prognostic factor for skin melanoma. *Am J Pathol* 147:1049–1056, 1996

Lugassy C, Eyden BP, Christensen L, Escande JP: Angio-tumoral complex in human malignant melanoma characterised by free laminin: ultrastructural and immuno-histochemical observations. *J Submicrosc Cytol Pathol* 29:19–28, 1997

Lugassy C, Dickersin GR, Christensen L, Karaoli T, Le Charpentier M, Escande JP, Barnhill RL: Ultrastructural and immunohistochemical studies of the periendothelial matrix in malignant melanoma: evidence for an amorphous matrix containing laminin. *J Cutan Pathol* 26:78–78, 1999a

Lugassy C, Shahsafaei A, Bonitz P, Busam KJ, Barnhill RL: Tumor microvessels in melanoma express the beta-2 chain of laminin. Implications for melanoma metastasis. *J Cutan Pathol* 26:222–226, 1999b