American Journal of Pathology, Vol. 133, No. 2, November 1988

The Prognostic Significance of Tumor Vascularity in Intermediate-Thickness (0.76–4.0 mm Thick) Skin Melanoma

A Quantitative Histologic Study

ANURAG SRIVASTAVA, MB, BS, PhD, FRCS,*
PETER LAIDLER, BM, BCh, DM, FRCPath,†
ROSALIND P. DAVIES,†
KIERAN HORGAN, MB, MCh, FRCS,* and
LESLIE E. HUGHES, MB, DS, FRCS, FRACS*

From the Departments of Surgery and Pathology,† University of Wales College of Medicine, Heath Park, Cardiff, United Kingdom*

The vascularity of 20 primary skin melanomas was assessed histologically. These cases were selected from patients with intermediate thickness melanomas (0.76–4.0 mm thick) treated surgically to provide two groups of ten patients. One group had no evidence of recurrence with a minimum follow-up of 9 years. The second group of ten patients developed locoregional or systemic metastasis under follow-up, and seven of these patients died of disseminated melanoma. Age, sex, Breslow's tumor thickness, and Clark's level of invasion were similar in the two groups. Vascular quantitation was carried out by image analysis after vascular definition by Ulex europaeus-I agglutinin staining. The percentage vascular area at the tumor base in the recurrence group was more than twice that in the recurrence-free group. This study suggests that increased vascularity at the tumor base may have prognostic significance in intermediate thickness melanomas. (Am J Pathol 1988, 133:419–423)

THE PROGNOSIS OF patients with primary, clinical stage I melanomas is best determined by the maximum tumor thickness.[1] In a subset of melanomas with tumor thickness between 0.76 and 3.99 mm, however, Day et al[2,3] reported that tumor thickness is less helpful in predicting the risk of distant metastasis and survival in an individual case. Accordingly, there is a need to explore further prognostic factors for this subset of skin melanomas. The present study investigates the prognostic significance of tumor vascularity in such intermediate-thickness (0.76–4.0 mm) melanomas.

Patients and Methods

There were 82 patients with an intermediate-thickness skin melanoma (0.76–4.0 mm thick) treated surgically in the authors' unit between 1971 and 1985. Forty-six patients remained disease free with a minimum follow-up period of 5 years (nonmetastatic group). In the other group, 36 patients developed metastases as follows: local recurrence, 10 patients; lymph node metastasis, 16 patients; systemic metastasis, 8 patients; local and systemic metastases, 1 patient; and lymph node and systemic metastases, 1 patient. This group was called the metastatic group. Ten patients from the nonmetastatic group were chosen to form matched pairs with another ten patients from the metastatic group to control for age, sex, and Breslow's tumor thickness. Because of the small number of patients studied it was not possible to match for the tumor site in all of them.

In the nonmetastatic group, patients remained free from metastasis for 111 months or more. In the metastatic group, five patients developed lymph node metastases and nine patients developed systemic spread. Seven of the ten patients in the metastatic group died

Supported by a grant from the Cancer Research Campaign, United Kingdom.

Accepted for publication July 11, 1988.

Address reprint requests to L. E. Hughes, University Department of Surgery, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, United Kingdom.

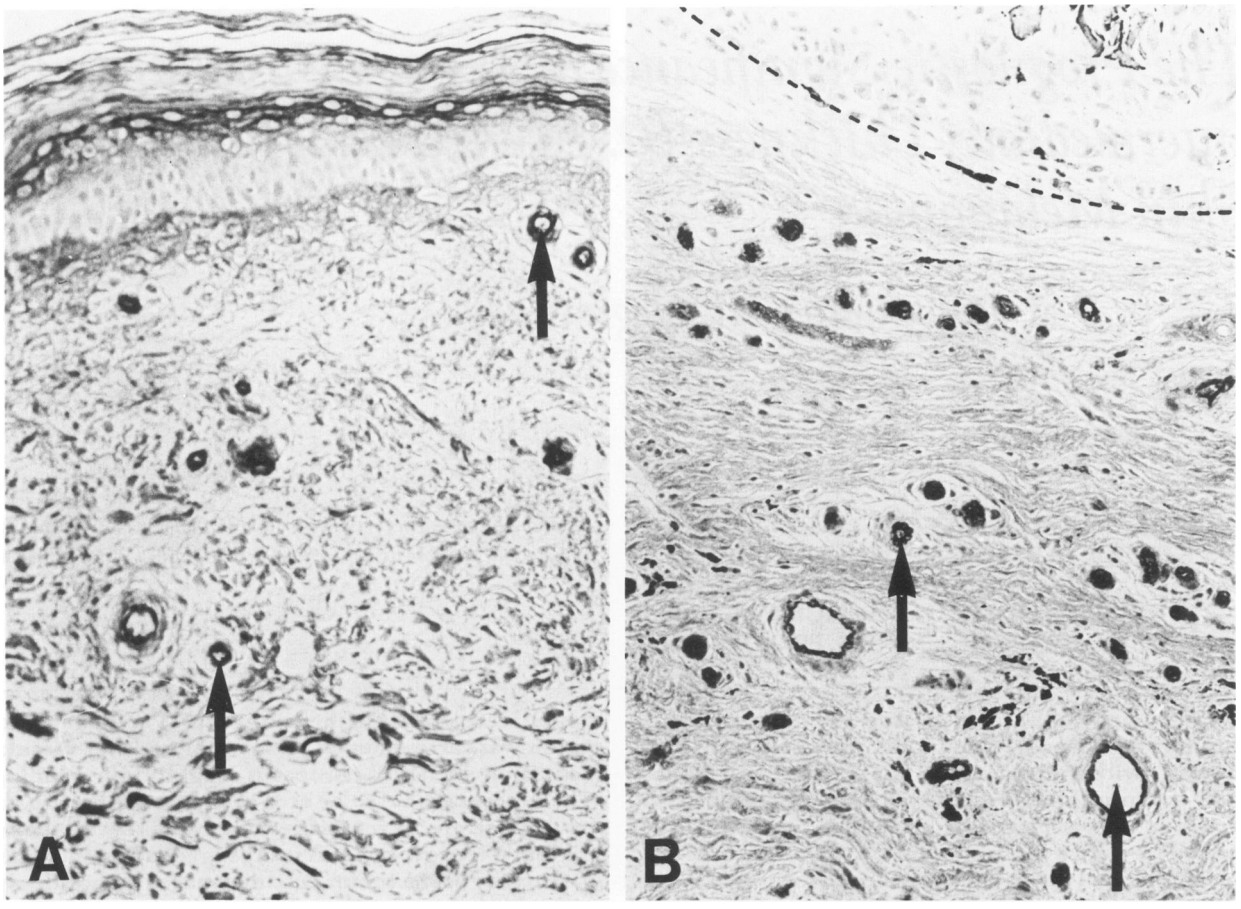

Figure 1—Ulex europaeus agglutinin 1 (UEA-1) peroxidase staining of vessels in histological sections of normal skin (**A**) and from the base of a melanoma (**B**). Vessels (arrows) are demonstrated by staining of the endothelial lining and are seen as circular or elliptical cross-sections. At the base of the melanoma, vessels are more plentiful than in normal skin, but are similar in size and shape in cross-section. The edge of the melanoma is marked by an interrupted line.

of disseminated melanoma. The primary lesion was excised with a 2–5-cm wide clearance depending on the clinical type of lesion. A lesion that was just palpable excised with 2 cm clearance and an overtly nodular tumor was excised with 3–5 cm clearance.[4] Five patients in the metastatic group underwent regional lymph node dissection at the time of primary treatment. Four of these cases had no histologic evidence of lymph node involvement and one patient had an involved lymph node on histologic examination. The follow-up is complete up to December 1986.

Methods

Four to five-micron thick sections were cut from the thickest part of the tumor including adjacent normal skin. These sections were stained with hematoxylin and eosin (H & E) for histologic evaluation and Ulex europaeus-I agglutinin conjugated with peroxidase (UEA-I) for the assessment of vascularity (Figure 1).[5]

The lectin-stained (UEA-I) sections were examined by an IBAS-2 semi-automatic image analysis system (Kontron Ltd, West Germany). The slides were coded and clinical information was not known to the observer at the time of image analysis. The lectin-stained slide was projected on the IBAS screen through a microscope with a magnification of ×160 or ×400, depending on the size of the tumor.

Using the automatic image analysis system, the following vascular parameters were computed: 1) Number of vessels per unit area of tissue cross-section (10^{-7} sq m); 2) Maximum diameter of vessels in 10^{-6} m (Max-D); and 3) Percentage vessel area (PVA). PVA was calculated as the percentage of whole field area occupied by the vessels. If vessels occupied 5% of the whole field, the PVA of that field was given a value of 5. All the blood vessels, irrespective of their size and shape, were included in the measurements. These measurements were made at three sites: at the tumor tissue, at the junctional zone between melanoma and underlying tissue, the tumor base, and at adjacent normal dermis.

Because tumors showed a heterogeneous distribution of blood vessels, an attempt was made to cover the whole tumor and tumor base area by multiple (be-

Table 1—Tumor Location

Site	Metastatic group	Nonmetastatic group
Head and neck	4	2
Upper limb	1	1
Lower limb	5	5
Back	-	2

tween 3 and 18 fields) field examination. The average of all the readings was taken. Nonparametric ranking tests were used for the statistical analysis.

Results

Age

The median age of patients in the nonmetastatic group was 54 years; in the metastatic group it was 56 years. This difference was not significant (Mann-Whitney U test, $P = 0.79$).

Sex

There were nine female and one male patients in the nonmetastatic group and seven female and three male patients in the metastatic group. The sex ratio in the two groups was not different on chi-square test, $P > 0.25$.

Site

The site of the melanomas in the two groups was to be as similar as possible (Table 1).

Maximum Tumor Thickness

The mean tumor thickness was 2.07 mm in the non-recurrence group (median, 2.25 mm; standard deviation, 0.78 mm). Melanomas in the metastatic group had a mean tumor thickness of 2.18 mm (median, 2.4 mm; standard deviation, 1.03 mm). The two groups had statistically similar thickness using the Mann-Whitney U test ($P = 0.76$).

Clark's Level of Invasion

Tables 2 and 3 show the distribution of different levels in the two groups of tumors. Half of the lesions in each group had invasion to the reticular dermis (level IV). Three tumors in the metastatic group and five tumors in the nonmetastatic group showed level III invasion.

Vascular Quantitation

The results of measurement of vessels in histologic sections from the tumor base are shown in Tables 2 and 3.

Number of Vessels per 10^{-7} sq m

There were 5.9 ± 2.8 vessels (mean ± 1 standard deviation) per unit area in the nonmetastatic group and 7.16 ± 3.5 vessels in the metastatic group. This difference was not statistically significant (Mann-Whitney, $P = 0.62$).

Maximum Vessel Diameter

The mean values for (Max-D) were 37.2 ± 7.6 μ in the nonmetastatic group and 49.5 ± 20.1 μ in the group with metastasis (not significant, Mann-Whitney, $P = 0.07$).

Percent Vessel Area

The percent vascularity in the no recurrence group was 1.57 ± 1.28% and that of the recurrence group was 4.62 ± 3.36%. This difference is significant (Mann-Whitney, $P = 0.025$).

The vascular parameters within the tumor showed no difference between the two groups. The number of vessels, Max-D and, PVA were all significantly higher at the tumor base region when compared with the corresponding values at the adjacent normal dermis in both groups of melanomas (Wilcoxon paired rank test, Table 4).

Discussion

The control of neovascularisation of tumors by tumor-derived growth factors is clearly an important biologic process in relation to tumor behavior. Although solid tumors vary considerably in their vascular response there appears to be a relationship between the degree of vascularity and the growth rate, at least in experimental tumors.[6] The present study tested the hypothesis that the biologic aggressiveness of a malignant growth is proportional to its vascularity. It has already been shown that *in situ* pre-invasive tumors are avascular and onset of angiogenesis ushers in a phase of rapid growth.[7] We have shown a relationship between melanoma thickness and vascularization as detected by Doppler ultrasound.[8] Once the angiogenesis has started, is the degree of vascularity related to the tumor growth rate, and more importantly, to the risk of metastasis in a human cancer?

This hypothesis has been tested by an objective assessment of tumor vascularity using UEA-I staining. This lectin has been shown to be a sensitive marker

Table 2—Vascular Quantitation in Melanomas with Metastasis

Tumor thickness (mm)	Clark's level of invasion	Primary site	Vascular parameters at tumour base: Number of vessels per 10^{-7} sq m	Maximum diameter 10^{-6} m	Percent vessel area	Site of metastasis	Survival (status) months
0.8	IV	Ear	5.9	47	2.7	Systemic	75 (dead)
0.95	II	Foot	3.4	25	0.44	Systemic	67 (alive)
1.3	III	Arm	2	100	7.3	Regional lymph nodes and systemic	79 (dead)
1.4	IV	Foot	7.3	57	7.0	Local, lymph nodes and systemic	59 (dead)
2.0	III	Leg	5.49	41	1.17	Regional lymph node	63 (alive)
2.8	III	Neck	10.5	43	4.14	Systemic	30 (dead)
2.8	IV	Thigh	5.9	40	1.96	Regional lymph nodes and systemic	48 (dead)
2.8	IV	Face	6.5	58	7.1	Systemic	39 (dead)
3.3	V	Leg	12	46	11	Systemic	8 (dead)
3.7	IV	Scalp	12.6	38	3.4	Systemic	110 (alive)
2.18*			7.16*	49.5*	4.6*		57.8*
1.03†			3.5†	20†	3.3†		28.5†
2.4‡			-‡	-‡	-‡		61‡

* Mean.
† 1 SD.
‡ Median.

for vascular endothelium. It stains both normal and tumor vessels.[9]

The detection of a significantly higher vascularity at the base of melanomas that were associated with metastasis suggests that the vascularity may have a prognostic significance independent of thickness.

Because of the small number of intermediate thickness melanomas in this series and the difficulty of matching for the Breslow's thickness, age, and sex, it was not possible to control for the site of the primary tumor in all the cases. Thus, there are slightly more cases of head and neck tumors in the metastatic group when compared with the nonmetastatic group. While this is a limiting factor of the study, the observation of higher vascularity of head and neck lesions may be a reason for the poorer prognosis of these melanomas.

Marasa and Tomasino[10] described the vascularity of 26 Clark's level III melanomas. An objective scoring system for vascular quantitation called microscopic angiogenesis grading system MAGS was used.[11] The MAGS was calculated by the histologic assessment of number of blood vessels per high power

Table 3—Vascular Quantitation in Melanomas without Metastasis

Tumor thickness (mm)	Clark's level of invasion	Primary site	Vascular parameters at tumor base: Number of vessels per 10^{-7} sq m	Maximum diameter 10^{-6} m	Percent vessel	Survival months
0.8	III	Leg	2.9	31	0.42	150
0.9	III	Sole (foot)	5.3	40	1.2	119
1.6	III	Sole (foot)	9.5	41	3.79	150
1.9	III	Arm	2.18	47	0.33	130
2.2	III	Back	6.0	37	1.3	124
2.3	IV	Back	1.9	31	0.23	123
2.3	IV	Leg	8.6	36	1.7	161
2.6	IV	Head	8.0	46	3.7	116
3.0	IV	Face	6.7	41	2.1	111
3.1	IV	Leg	8.4	22	1.01	132
2.07*			5.94*	37.2*	1.57*	131*
0.78†			2.8†	7.6†	1.28†	16.6†
2.25‡			-‡	-‡	-‡	127‡

* Mean.
† 1 SD.
‡ Median.

Table 4—Comparison of Tumor Base Vascularity with Normal Dermis

	Tumor base	Normal dermis
Metastatic group		
Number of vessels per 10^{-7} m	7.16 ± 3.5	4.2 ± 2.1 $P < 0.02$
Maximum diameter in 10^{-6} m	49.5 ± 20	36.4 ± 12.9 $P < 0.001$
Percent vessel area	4.6 ± 3.3	0.88 ± 0.5 $P = 0.005$
Nonmetastatic group		
Number of vessels per 10^{-7} sq m	5.94 ± 2.8	3.1 ± 1.78 $P < 0.001$
Maximum diameter in 10^{-6} m	37.2 ± 7.6	24.7 ± 6.57 $P < 0.001$
Percent vessel area	1.57 ± 1.28	0.41 ± 0.38 $P < 0.001$

All values as mean ± 1 standard deviation. *P* values by Wilcoxon Paired Rank Test.

field, the degree of endothelial cell hyperplasia, and the grade of endothelial cell atypia. A high score of MAGS indicated high vascularity. None of the 11 patients with melanomas that had a MAGS score of over 30 survived for 5 years, whereas patients with melanomas of a MAGS score of less than 30 had a 5-year survival of 86%. Marasa and Tomasino also found the vascularity to be maximum at the tumor edge.[10]

The interesting biologic phenomenon of angiogenesis has drawn the attention of many research workers. It seems that there are many angiogenic factors and some of the most investigated growth factors responsible for tumor angiogenesis are endothelial cell growth factor,[12] α transforming growth factor,[13] β transforming growth factor,[14] and acidic and basic types of fibroblast growth factors.[15] Besides being growth regulatory peptides for tumor cells, these factors also express a mitogenic effect on the endothelial cells and help in the regulation of blood vessel growth. A rapidly growing tumor of high biologic aggressiveness probably has high activity of these growth factors that evoke an intense angiogenic response.

Thus, in theory, biologic behavior and vascularity of a tumor should go hand in hand. This study has provided some preliminary evidence that these considerations apply in the development of metastatic potential in human melanomas. The study now needs expansion and inclusion of a larger number of patients to prove any prognostic significance of vascularity independent of other variables, such as tumor thickness.

References

1. Breslow A: Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma. Ann Surg 1970, 172:902–908
2. Day CL Jr, Mihm MC Jr, Sober AJ, Harris MN, Kopf AW, Fitzpatrick TB, Lew RA, Harrist TJ, Golomb FM, Postel A, Hennessey P, Gumport SL, Raker JW, Malt RA, Cosimi AB, Wood WC, Roses DF, Gorstein F, Rigel D, Friedman RJ, Mintzis MM: Prognostic factors for melanoma patients with lesions 0.76–1.69 mm in thickness, an appraisal of "thin" level IV lesions. Ann Surg 1982, 195:30–34
3. Day CL Jr, Mihm MC Jr, Lew RA, Harris MN, Kopf AW, Fitzpatrick TB, Harrist TJ, Golomb FM, Postel A, Hennessey P, Gumport SL, Raker JW, Malt RA, Cosimi AB, Wood WC, Roses DF, Gorstein F, Rigel D, Friedman RJ, Mintzis MM, Sober AJ: Prognostic factors for patients with clinical stage I melanoma of intermediate thickness (1.51–3.99 mm): A conceptual model for tumor growth and metastasis. Ann Surg 1982, 195:35–43
4. Taylor BA, Hughes LE, Williams GT: Improving prognosis for malignant melanoma in Britain. Br J Surg 1984, 71:950–953
5. Walker RA: Ulex europeus-I peroxidase as a marker of vascular endothelium: Its application in routine histopathology. J Pathol 1985, 146:123–127
6. Rofstad EK: Growth and vascular structure of human melanoma xenografts. Cell Tissue Kinet 1984, 17:91–101
7. Folkman J, Klagsbrun M: Tumor angiogenesis: Effect on tumor growth and immunity, Fundamental Aspects of Neoplasia. Edited by AA Gottlieb, OJ Plescia, DHL Bishop. New York, Springer-Verlag, 1975: 401–412
8. 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
9. Miettinen M, Holthofer H, Lehto V-P, Miettinen A, Virtanen I: Ulex europaeus-I lectin as a marker for tumors derived from endothelial cells. Am J Clin Pathol 1983, 79:32–36
10. Marasa L, Tomasino RM: Melanoma cutaneo II. Proposta di un nuovo parametro prognostico: La valutazione dell'angiogenesi neoplastica. Pathologica 1983; 75: 353–365
11. Brem S, Cotran R, Folkman J: Tumor angiogenesis: A quantitative method for histologic grading. J Natl Cancer Inst 1972, 48:347–356
12. Maciag T, Mehiman T, Friesel R, Screiber AB: Heparin binds endothelial cell growth factor, the principal endothelial cell mitogen in bovine brain. Science (Wash DC) 1984; 225:932–935
13. Derynck R: Type alpha transforming growth factor: A direct angiogenic molecule? Symposium on Angiogenesis. National Institute of Health, Bethesda, MD, June 3–4, 1987
14. Sporn M: Type beta transforming growth factor: An indirect angiogenic molecule? Symposium on Angiogenesis. National Institute of Health, Bethesda, MD, June 3–4, 1987
15. Lobb RR, Fett JW: Purification of two distinct growth factors from bovine neural tissue by heparin affinity chromatography. Biochemistry 1984; 23:6295–6299

Acknowledgment

The authors thank Miss Mary Ryan for her help in preparing the manuscript.