Association of Tumor Angiogenesis With Bone Marrow Micrometastases in Breast Cancer Patients

*Stephen B. Fox, Russel D. Leek, Judith Bliss, Janine L. Mansi, Barry Gusterson, Kevin C. Gatter, Adrian L. Harris**

Background and Purpose: **The microscopic detection of tumor cells (micrometastases) in bone marrow and the extent of blood vessel formation (angiogenesis) in primary tumor specimens are recognized as independent prognostic markers in patients with breast cancer. Since micrometastases occur as a consequence of interaction between the neoplastic cells and the tumor neovasculature, we have examined the relationship between these markers to determine whether the degree of angiogenesis is related to the presence of micrometastases.** ***Methods:*** **Micrometastases were identified in bone marrow aspirates collected from multiple sites in 214 breast cancer patients prior to surgery (mastectomy or lumpectomy). Tumor cells were detected through an examination of epithelial membrane antigen expression and an analysis of cell morphology. Tumor vascularity was graded semiquantitatively or quantitatively (Chalkley point count) after immunohistochemical staining of the CD31 antigen expressed by the endothelial cells. The reproducibility and accuracy of the vascular grading were validated by use of kappa statistics. Associations between micrometastases and clinicopathologic characteristics, including angiogenesis, were examined using chi-squared and logistic regression techniques. All tests of statistical significance were two-sided.** ***Results:*** **Of the 214 patients, 42 (20%) were positive for bone marrow micrometastases and 75 (35%) had tumors of high vascular grade. There was 86% agreement between vascular grades assessed twice for 35 tumors (kappa statistic = 0.66); for 22 evaluated tumors, there was absolute concordance between vascular grade and Chalkley point count. There were significant positive associations between tumor angiogenesis and micrometastasis (*P* = .01), tumor grade (*P* = .003), and estrogen receptor expression (*P* = .007); however, no significant associations were observed with tumor size (*P* = .9), lymph node status (*P* = .33), vascular invasion (peritumoral blood or lymph vessels) (*P* = .9), menopausal status (*P* = .17), or age (*P* = .12). Adjusting for confounding factors, multivariate analysis showed that only tumor angiogenesis (odds ratio = 2.7; *P* = .016) and vascular invasion (odds ratio = 2.7; *P* = .012) were significant determinants for the presence of micrometastases.** ***Conclusions:*** **This study suggests that an assessment of tumor angiogenesis and vascular invasion gives a reliable indication of the likelihood of the presence of bone marrow micrometastases in patients with breast cancer and that both processes contribute to metastases. [J Natl Cancer Inst 1997;89:1044-9]**

Despite apparent curative surgery, a large proportion of lymph node-negative breast cancer patients will die of metastatic disease that is undetected by conventional methods at presentation. In some studies, bone marrow micrometastases identified by immunohistochemistry at primary surgery have predicted disease progression and have been associated with a significant reduction in relapse-free and overall survival in several tumor types, including breast carcinomas (*1-8*). Therefore, it has been suggested that examination for bone marrow micrometastasis in breast cancer patients would be useful as a prognostic marker and would help to define and monitor patient groups who are at high risk and who would benefit from adjuvant therapy.

Angiogenesis, the development of new vessels from the existing vasculature, is essential for tumor growth (*9*). The angio-

**Affiliations of authors:* S. B. Fox, K. C. Gatter (Department of Cellular Science), R. D. Leek, A. L. Harris (Imperial Cancer Research Fund Molecular Oncology Laboratory), University of Oxford, John Radcliffe Hospital, U.K.; J. Bliss (Section of Epidemiology), B. Gusterson (Haddow Laboratories), Institute of Cancer Research, Sutton, Surrey, U.K.; J. L. Mansi, Department of Medical Oncology, St. George's Hospital, London, U.K.

Correspondence to: Professor Adrian L. Harris, Imperial Cancer Research Fund Molecular Oncology Laboratory, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, U.K.

See ''Notes'' following ''References.''

genic activity of a tumor as assessed by microvessel density has been shown to be a powerful independent prognostic factor in many tumor types, including breast carcinoma (*10-12*). Because interaction between this neovasculature and neoplastic cells results in metastasis, we have examined the relationship between angiogenesis and bone marrow micrometastases in a series of breast cancer patients to determine whether the extent of angiogenesis is related to the presence of bone marrow micrometastases.

Subjects and Methods

Patients. From a cohort of female patients with primary breast cancer in which the relationship between bone marrow micrometastases and other clinicopathologic features had been previously reported (*13*), 214 with data and material were available for use for this study. This series of patients is, however, different from those studied by our group for quantitative assessment of angiogenesis. The patient group had a median age of 61 years (range, 26-85 years), had been previously treated by mastectomy or lumpectomy (63 and 151 patients, respectively) depending on the size and site of the tumor, and had been administered adjuvant hormonal and chemotherapy treatment (36 and 52 patients, respectively). Written informed consent was obtained from the patients, and the protocol was approved by the Hospital Ethical Committee. Estrogen receptor expression was measured by the dextran-coated charcoal method (*14*). Vascular invasion was defined as the presence of tumor cells in blood and/or lymph vessels in or around the tumor. The clinicopathologic characteristics of the patients are summarized in Table 1.

Tumor angiogenesis. Tumor vascularity was measured in the primary tumors of 214 patients by vascular grading. Sections of tumors were cut onto slides coated with silane (Sigma Chemical Co., Poole, Dorset, U.K.) and predigested with 12.5 mg of protease type XXIV (Sigma Chemical Co.) per 100 mL phosphate-buffered saline for 20 minutes at 37 °C before application of the primary mouse anti-CD31 antibody JC70 (Dako Ltd., High Wycombe, Bucks., U.K.) at 16 μg/mL and labeling by the alkaline phosphatase anti-alkaline phosphatase method (*15*). Tumors were classified into high and low/medium categories as previously described (*16*). Briefly, tumor sections were scanned at low power (×40-100) for the three areas of highest vascularity under a conference microscope by two observers who were blinded to the clinicopathologic data obtained from the patients. These sections were then examined at high power (×250-400) and placed into one of the two categories discussed above by use of a semiquantitative subjective score that has been previously shown to be significantly correlated with microvessel density and Chalkley point counting (*16*). On two separate occasions, we reassessed 35 cases to measure reproducibility; we also assessed 22 tumors (from 10% of the patient series) by the Chalkley count to ensure quantitative accuracy (*16*). The Chalkley count uses an eyepiece with random dots, and the score is made by counting the coincidence of vessels with eyepiece dots. This technique provides a measure of area occupied by blood vessels. The cutoff of high versus low/medium categories in the statistical analysis was used and in previous studies, as in this one, was shown to group patients into thirds (*16*). This procedure, therefore, avoids assumptions about relationships between tumor vascularity and other variables.

Bone marrow micrometastases. Approximately 16 mL of bone marrow aspirated from eight sites immediately prior to surgery was examined for micrometastasis. Bone marrow was prepared as previously described and stained with antibodies to epithelial membrane antigen (*17*). Patients were considered positive for micrometastasis only if cells expressed epithelial membrane antigen and were morphologically malignant.

Statistical analysis. Chi-squared tests were used to examine the relationship between angiogenesis and clinicopathologic variables. Logistic regression techniques with *P* values based on the likelihood ratio statistic were used to evaluate the role of angiogenesis as a predictor of micrometastasis after adjusting for factors known to be determinants of an individual's risk of micrometastases at diagnosis. Only those patient–tumor variables previously found to be associated with micrometastasis in the complete cohort of 350 patients were considered

Table 1. Contingency table comparing angiogenesis with the clinicopathologic characteristics of breast cancer patients*

Characteristic	Angiogenesis		Total No.	Statistical analysis†
	Low	High		
Age, y				
<50	31	23	54	$\chi^2 = 2.48$; df = 1; $P = .12$ (trend)
50-64	51	29	80	
⩾65	57	23	80	
Lymph node status				
Negative	65	31	96	$\chi^2 = 0.96$; df = 1; $P = .33$
Positive	61	39	100	
Tumor size, cm				
⩽2	65	37	102	$\chi^2 = 0.00$; df = 1; $P = .9$
>2	64	36	100	
Vascular invasion				
Positive	58	32	90	$\chi^2 = 0.18$; df = 1; $P = .9$
Negative	81	43	124	
Grade (*42*)				
I	33	11	44	$\chi^2 = 8.78$; df = 1; $P = .003$ (trend)
II	71	32	103	
III	22	27	49	
Micrometastases				
Positive	20	22	42	$\chi^2 = 6.65$; df = 1; $P = .01$
Negative	119	53	172	
ER expression,‡ fmol/mg protein				
<15	100	41	141	$\chi^2 = 7.48$; df = 1; $P = .007$
⩾15	34	32	66	
Menopausal status				
Premenopausal	27	19	46	$P = .17$
Postmenopausal	104	50	154	

*Where total numbers do not equal 214, data are unavailable.
†df = degrees of freedom. All statistical tests were two-sided.
‡ER = estrogen receptor.

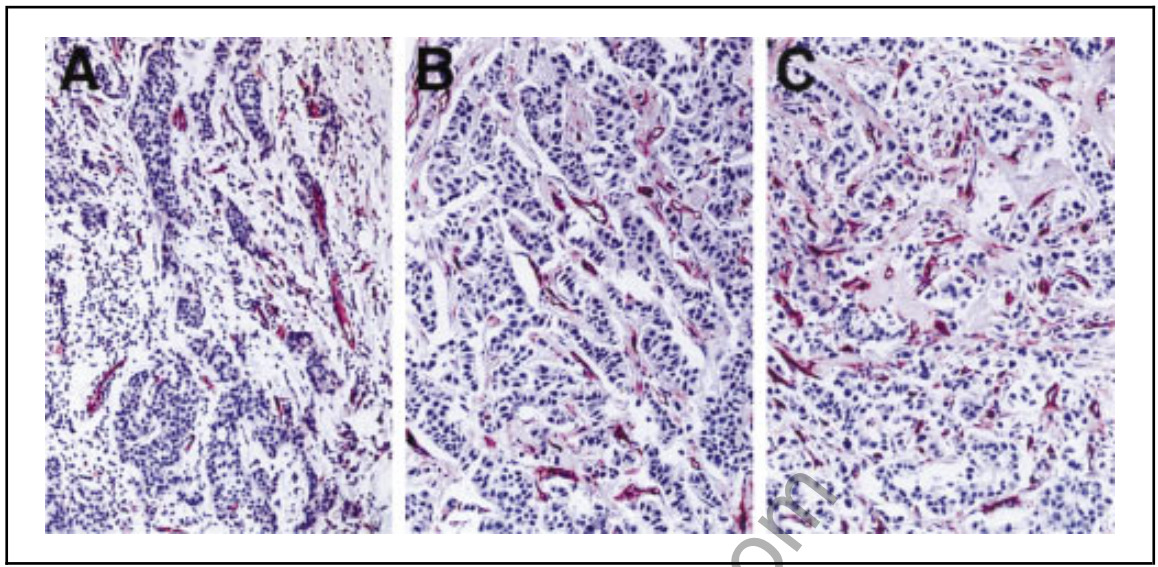

Fig. 1. Three invasive ductal carcinomas stained with anti-CD31 antibody JC70, demonstrating low **(A),** medium **(B),** and high **(C)** vascular grades (original magnification ×250).

(*13*), i.e., tumor size, lymph node status, vascular invasion, and estrogen receptor expression. All statistical tests were two-sided.

Results

The intensity of staining in endothelial cells in individual tumors was fairly homogeneous, but the vascularity of tumors differed such that 139 tumors (65%) were scored as low/medium vascular grade and 75 were scored as high (35%) (Fig. 1). In 35 tumors that were evaluated twice, there was 86% agreement between the initial and the repeat vascular grading (kappa statistic = 0.66). The discrepancies were coded according to the initial score. There was absolute concordance in 22 tumors between vascular grade and Chalkley count by use of a cutoff of ⩾7 [a value previously demonstrated to be significantly associated with a poor prognosis (*16*)]. Of the total of 214 tumors, 42 (20%) were positive for bone marrow micrometastasis and 172 (80%) were negative.

There was a significant positive association between angiogenesis at the primary tumor site and micrometastasis ($P = .01$), tumor grade ($P = .003$), and estrogen receptor (ER) expression ($P = .007$), but no significant association was observed with tumor size ($P = .9$), lymph node status ($P = .33$), lymphatic and/or blood vessel invasion ($P = .9$), menopausal status ($P = .17$), or age ($P = .12$) (Table 1).

Previous analyses in the full patient cohort have demonstrated that lymph node involvement, tumor size, vascular invasion, and ER expression are each associated with micrometastases (*13*). To determine whether angiogenesis retains its relationship to micrometastasis, after adjusting for these particular confounding factors, a multivariate analysis was performed that gave an estimate of the odds ratio (unadjusted odds ratio = 2.5; 95% confidence interval = 1.2-4.9) similar to the unadjusted value. Since in a univariate analysis vascular invasion has the strongest association with micrometastases (*13*), this was the first factor used to generate the multivariate model (Table 2). After adjustment for this variable, lymph node status and tumor size no longer exhibited a statistically significant association with micrometastasis. Therefore, most of the effect of lymph node involvement and tumor size on the risk of micrometastases appears to be explained by their associations with vascular invasion. No interaction or synergy was observed between vascular invasion and angiogenesis in patients positive for micrometastasis (Table 3). In a new model adjusting only for the factors that had $P<.1$ in the full multivariate analysis (Table 2), angiogenesis retained its predictive power (Table 4).

Discussion

This study has examined the association between primary tumor angiogenesis and the presence of bone marrow microme-

Table 2. Multivariate analysis examining relationship between micrometastases and other clinicopathologic variables*

Variable	Odds ratio	95% confidence interval	Two-sided *P*
Vascular invasion			
Negative	1.0		
Positive	2.8	1.2-6.7	.018
Grade (*42*)			
I	1.0		
II	3.7	0.8-17.9	.1
III	2.3	0.4-12.7	.35
Angiogenesis			
Low	1.0		
High	2.8	1.2-6.4	.015
ER expression,† fmol/mg protein			
⩾15	1.0		
<15	2.2	0.9-5.1	.08
Lymph node status			
Negative	1.0		
Positive	1.04	0.4-2.5	.93
Size, cm			
⩽2	1.0		
>2	1.4	0.6-3.4	.44

*Multivariate analysis (logistic regression) of 177 breast cancer patients for whom complete data are available. We examined the relationship between micrometastases and other clinicopathologic variables that have been previously shown to be significantly associated with micrometastases (*13*) and angiogenesis.

†ER = estrogen receptor.

Table 3. Number and percentage of patients positive for micrometastases for each combination of angiogenesis (low/medium or high) and vascular invasion (positive or negative)

	Angiogenesis*	
Vascular invasion	Low/medium	High
Positive	13/58 (22)	14/32 (44)
Negative	7/81 (9)	8/43 (19)

*Values in columns = number of patients positive for micrometastases/total number of patients (%).

tastasis together with conventional clinicopathologic characteristics in a series of breast cancer patients. We used a vascular grading system (*12*,*16*) to quantify tumor angiogenesis and epithelial membrane antigen in conjunction with morphology to identify micrometastasis, the latter giving a positive identification rate within the range observed in other studies, i.e., 4%-48% (*3*,*6*,*18*-*22*).

Only tumor angiogenesis and vascular invasion gave independent information about the likelihood of micrometastasis by use of a multivariate analysis after adjusting for confounding variables. Furthermore, the observation that tumor angiogenesis was not significantly associated with vascular invasion is consistent with our current understanding of tumor dissemination and supports the concept that cooperating, separate pathways are required for tumor metastasis.

The significant positive association between high angiogenesis and the presence of bone marrow micrometastasis is likely to be partly due to the larger endothelial surface area with which the tumor cells can interact. This theory is supported by the positive association observed between microvessel density and the number of intraoperative, circulating tumor cells in breast cancer patients (*23*). Although the prognostic significance of shed tumor cells is unknown (*23*), the positive association between high tumor angiogenesis and both micrometastasis and shedding suggests that the poor prognostic outcome in this patient group is likely related to a combination of enhanced tumor growth and access to the circulation.

Table 4. Multivariate analysis of 189 breast cancer patients: relationship between micrometastases and angiogenesis after adjusting for grade, estrogen receptor (ER) expression, and vascular invasion*

Variable	Odds ratio	95% confidence interval	Two-sided *P*
Grade (*42*)			
I	1.0		
II	4.9	1.1-22.8	.04†
III	2.5	0.5-13.2	.3†
Angiogenesis			
Low	1.0		
High	2.7	1.2-5.9	.016
ER expression, fmol/mg protein			
⩾15	1.0		
<15	2.0	0.9-4.7	.1
Vascular invasion			
Negative	1.0		
Positive	2.7	1.3-6.0	.012

*Multivariate analysis of data from 189 patients for whom complete data were available. Unadjusted odds ratio for angiogenesis: 2.5 (95% confidence interval = 1.2-4.9).

†These unusual estimates probably reflect the relatively small sample.

Nevertheless, as indicated above, the presence of tumor vessels alone is not sufficient for metastasis to occur. The malignant cells must cross the specialized tumor stroma and interact with the neovasculature to access the circulation. There is considerable evidence demonstrating that, for a tumor to acquire a metastatic phenotype, it must modulate a variety of genes involved in stromal invasion (*24*). We have shown the requirement for vascular invasion to facilitate tumor metastasis in mouse xenograft models. The transfection of breast tumor cell lines with genes promoting angiogenesis, such as vascular endothelial growth factor, increases tumor vascularity and growth but does not alter the metastatic rate (*25*). In contrast, the transfection of genes enhancing stromal proteolysis, e.g., components of the urokinase plasminogen activator system, results in an increase in metastasis (*26*). Thus, both pathways are required to generate metastasis, one providing the framework and the other providing a mechanism to penetrate this structure. Indeed, in breast cancer patients, the urokinase plasminogen activator and angiogenesis have each been shown to be independent prognostic factors (*11*,*12*,*27*). Since similar protease systems are used for both invasion and angiogenesis (*28*), it is likely that patients with tumors with high vascularity and prominent vascular invasion might benefit from protease inhibitors as adjuvant therapy.

Once tumor cells have gained access to the circulation, they must colonize distant sites. The concept of dormancy is well recognized, and in an attempt to predict the likelihood of progression, the expression of oncogenes (*29*), adhesion molecules (*30*), members of the urokinase plasminogen activator system (*31*,*32*), major histocompatibility complex molecules (*33*,*34*), and proliferation index (*29*) have been studied. Although these studies are still in their infancy, there are data to suggest that these tumor cell characteristics will not differentiate between dormant and active micrometastases (*35*,*36*) and that the net growth rate and progression of micrometastases are determined by the angiogenic phenotype of the tumor. When angiogenesis is suppressed, the tumor cell proliferation rate matches the apoptotic rate (*35*), resulting in dormant tumors that are unable to grow beyond 2-3 mm in diameter, the diffusion range of the existing vessels (*9*). When the dormant micrometastasis assumes an angiogenic phenotype and establishes a vascular network, the apoptotic rate is reduced without a concomitant effect on tumor cell proliferation, enabling net tumor growth (*35*-*37*). Thus, a more successful strategy to predict clinical progression might be to measure the angiogenic activity of tumor metastasis, the net result of the balance between inhibitors and stimulators. By measurement of the levels of these inhibitors and stimulators in micrometastases, the serum (*38*,*39*), and/or urine (*40*,*41*), it might be possible to establish when this switch has occurred and to predict when micrometastases will become clinically important.

In the meantime, this study suggests that the assessment of tumor vascularity and vascular invasion gives a reliable indication of the likelihood of the presence of micrometastases and that

the extent of angiogenesis might be useful as a surrogate marker for the capacity of a tumor to metastasize.

References

(*1*) Pantel K, Izbicki JR, Angstwurm M, Braun S, Passlick B, Karg O, et al. Immunocytological detection of bone marrow micrometastasis in operable non-small cell lung cancer. Cancer Res 1993;53:1027-31.

(*2*) Schlimok G, Funke I, Pantel K, Strobel F, Lindemann F, Witte J, et al. Micrometastatic tumour cells in bone marrow of patients with gastric cancer: methodological aspects of detection and prognostic significance. Eur J Cancer 1991;27:1461-5.

(*3*) Cote RJ, Rosen PP, Lesser ML, Old LJ, Osborne MP. Prediction of early relapse in patients with operable breast cancer by detection of occult bone marrow micrometastases. J Clin Oncol 1991;9:1749-56.

(*4*) Lindemann F, Schlimok G, Dirschedl P, Witte J, Riethmuller G. Prognostic significance of micrometastatic tumour cells in bone marrow of colorectal cancer patients. Lancet 1992;340:685-9.

(*5*) Schlimok G, Lindemann F, Holzmann K, Witte J, Renner D, Riethmuller G. Prognostic significance of disseminated tumor cells detected in bone marrow of patients with breast and colorectal cancer: a multivariate analysis [abstract]. Proc ASCO 1992;11:102.

(*6*) Osborne MP, Rosen PP. Detection and management of bone marrow micrometastases in breast cancer. Oncology (Huntingt) 1994;8:25-31.

(*7*) Diel IJ, Kaufmann M, Goerner R, Costa SD, Kaul S, Bastert G. Detection of tumor cells in bone marrow of patients with primary breast cancer: a prognostic factor for distant metastasis. J Clin Oncol 1992;10: 1534-9.

(*8*) Thorban S, Roder J, Nekarda H, Funk A, Siewert JR, Pantel K. Immunocytochemical detection of disseminated tumor cells in the bone marrow of patients with esophageal carcinoma. J Natl Cancer Inst 1996;88: 1222-7.

(*9*) Folkman J. What is the evidence that tumors are angiogenesis dependent? [editorial] J Natl Cancer Inst 1990;82:4-6.

(*10*) Bosari S, Lee AK, DeLellis RA, Wiley BD, Heatley GJ, Silverman ML. Microvessel quantitation and prognosis in invasive breast carcinoma. Hum Pathol 1992;23:755-61.

(*11*) Horak ER, Leek R, Klenk N, LeJeune S, Smith K, Stuart N, et al. Angiogenesis, assessed by platelet/endothelial cell adhesion molecule antibodies, as indicator of node metastases and survival in breast cancer. Lancet 1992; 340:1120-4.

(*12*) Weidner N, Folkman J, Pozza F, Bevilacqua P, Allred EN, Moore DH, et al. Tumor angiogenesis: a new significant and independent prognostic indicator in early-stage breast carcinoma [see comments]. J Natl Cancer Inst 1992;84:1875-87.

(*13*) Mansi JL, Easton D, Berger U, Gazet JC, Ford HT, Dearnaley D, et al. Bone marrow micrometastases in primary breast cancer: prognostic significance after 6 years' follow-up. Eur J Cancer 1991;27:1552-5.

(*14*) Nicholson S, Sainsbury JR, Needham GK, Chambers P, Farndon JR, Harris AL. Quantitative assays of epidermal growth factor receptor in human breast cancer: cut-off points of clinical relevance. Int J Cancer 1988;42: 36-41.

(*15*) Gatter KC, Falini B, Mason D. The use of monoclonal antibodies in histopathological diagnosis. In: Anthony PP, MacSween RN, editors. Recent advances in histopathology. London: Churchill-Livingstone, 1984:35-67.

(*16*) Fox SB, Leek RD, Weekes MP, Whitehouse RM, Gatter KC, Harris AL. Quantitation and prognostic value of breast cancer angiogenesis: comparison of microvessel density, Chalkley count, and computer image analysis. J Pathol 1995;177:275-83.

(*17*) Dearnaley DP, Sloane JP, Ormerod MG, Steele K, Coombes RC, Clink HM, et al. Increased detection of mammary carcinoma cells in marrow smears using antisera to epithelial membrane antigen. Br J Cancer 1981; 44:85-90.

(*18*) Porro G, Menard S, Tagliabue E, Orefice S, Salvadori B, Squicciarini P, et al. Monoclonal antibody detection of carcinoma cells in bone marrow biopsy specimens from breast cancer patients. Cancer 1988;61: 2407-11.

(*19*) Kirk SJ, Cooper GG, Hoper M, Watt PC, Roy AD, Odling-Smee W. The prognostic significance of marrow micrometastases in women with early breast cancer. Eur J Surg Oncol 1990;16:481-5.

(*20*) Salvadori B, Squicciarini P, Rovini D, Orefice S, Andreola S, Rilke F, et al. Use of monoclonal antibody MBr1 to detect micrometastases in bone marrow specimens of breast cancer patients. Eur J Cancer 1990;26: 865-7.

(*21*) Schlimok G, Funke I, Holzmann B, Gottlinger G, Schmidt G, Hauser H, et al. Micrometastatic cancer cells in bone marrow: in vitro detection with anti-cytokeratin and in vivo labeling with anti-17-1A monoclonal antibodies. Proc Natl Acad Sci U S A 1987;84:8672-6.

(*22*) Ellis G, Ferguson M, Yamanaka E, Livingston RB, Gown AM. Monoclonal antibodies for detection of occult carcinoma cells in bone marrow of breast cancer patients. Cancer 1989;63:2509-14.

(*23*) McCulloch P, Choy A, Martin L. Association between tumour angiogenesis and tumour cell shedding into effluent venous blood during breast cancer surgery. Lancet 1995;346:1334-5.

(*24*) MacDonald NJ, Steeg PS. Molecular basis of tumour metastasis. Cancer Surv 1993;16:175-99.

(*25*) Zhang HT, Craft P, Scott PA, Ziche M, Weich HA, Harris AL, et al. Enhancement of tumor growth and vascular density by transfection of vascular endothelial cell growth factor into MCF-7 human breast carcinoma cells. J Natl Cancer Inst 1995;87:213-9.

(*26*) Xing RH, Rabbani SA. Overexpression of urokinase receptor in breast cancer cells results in increased tumor invasion, growth and metastasis. Int J Cancer 1996;67:423-9.

(*27*) Grondahl-Hansen J, Christensen IJ, Rosenquist C, Brunner N, Mouridsen HT, Dano K, et al. High levels of urokinase-type plasminogen activator and its inhibitor PAI-1 in cytosolic extracts of breast carcinomas are associated with poor prognosis. Cancer Res 1993;53:2513-21.

(*28*) Fox SB, Gatter KC, Harris AL. Tumour angiogenesis. J Pathol 1996;179: 232-7.

(*29*) Pantel K, Schlimok G, Braun S, Kutter D, Lindemann F, Schaller G, et al. Differential expression of proliferation-associated molecules in individual micrometastatic carcinoma cells. J Natl Cancer Inst 1993;85:1419-24.

(*30*) Habets L, Albrecht S, Weinberg R, Essers U, Nagel K, Bary S, et al. No expression of CD44v5/v6 on cytokeratin positive cells in bone marrow of patients with colorectal tumors. In: First International Symposium on Minimal Residual Cancer. J Hematother 1996;5:564.

(*31*) Gruetzner K, Babic R, Jauch K, Schildberg F, Heiss M. uPA-receptor as a potential characteristic of metastatic phenotypes of disseminated tumor cells in bone marrow. In: First International Symposium on Minimal Residual Cancer. J Hematother 1996;5:563.

(*32*) Untch M, Funke I, Nestle-Kramling C, Konecny G, Bauerfeind I, Hepp H. Correlation of different prognostic factors in the metastatic cascade of primary breast cancer patients. In: First International Symposium on Minimal Residual Cancer. J Hematother 1996;5:569.

(*33*) Pantel K, Schlimok G, Kutter D, Schaller G, Genz T, Wiebecke B, et al. Frequent down-regulation of major histocompatibility class I antigen expression on individual micrometastatic carcinoma cells. Cancer Res 1991; 51:4712-5.

(*34*) Pantel K, Schlimok G, Angstwurm M, Passlick B, Izbicki JR, Johnson JP, et al. Early metastasis of human solid tumours: expression of cell adhesion molecules. Ciba Found Symp 1995;189:157-70.

(*35*) Holmgren L, O'Reilly MS, Folkman J. Dormancy of micrometastases: balanced proliferation and apoptosis in the presence of angiogenesis suppression. Nat Med 1995;1:149-53.

(*36*) Murray C. Tumour dormancy: not so sleepy after all. Nat Med 1995;1: 117-8.

(*37*) O'Reilly MS, Holmgren L, Chen C, Folkman J. Angiostatin induces and sustains dormancy of human primary tumours in mice. Nat Med 1996;2: 689-92.

(*38*) Fujimoto K, Ichimori Y, Kakizoe T, Okajima E, Sakamoto H, Sugimura T, et al. Increased serum levels of basic fibroblast growth factor in patients with renal cell carcinoma. Biochem Biophys Res Commun 1991;180:386-92.

(*39*) Kondo S, Asano M, Matsuo K, Ohmori I, Suzuki H. Vascular endothelial growth factor/vascular permeability factor is detectable in the sera of tumor-bearing mice and cancer patients. Biochim Biophys Acta 1994;1221: 211-4.

(*40*) Nguyen M, Watanabe H, Budson AE, Richie JP, Hayes DF, Folkman J.

Elevated levels of an angiogenic peptide, basic fibroblast growth factor, in the urine of patients with a wide spectrum of cancers. J Natl Cancer Inst 1994;86:356-61.

(*41*) O'Brien TS, Smith K, Cranston D, Fuggle S, Bicknell R, Harris AL. Urinary basic fibroblast growth factor in patients with bladder cancer and benign prostatic hypertrophy. Br J Urol 1995;76:311-4.

(*42*) Elston CW. Grading of invasive carcinoma of the breast. In: Page DL, Anderson TJ, editors. Diagnostic histopathology of the breast. Edinburgh: Churchill-Livingstone, 1987:300-11.

Notes

Present address: S. B. Fox, Department of Anatomical Pathology, Christchurch Hospital, New Zealand.

Supported in part by The Ludwig Institute for Cancer Research.

We thank Professor Munro Neville for his continuing support. We also thank Ann Ward for assisting with the micrometastasis database.

Manuscript received December 10, 1996; revised April 22, 1997; accepted May 7, 1997.