

Prognostic Importance of Glomeruloid Microvascular Proliferation Indicates an Aggressive Angiogenic Phenotype in Human Cancers¹

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Abstract

We evaluated the presence of glomeruloid microvascular proliferations (GMPs) in 723 patients with melanomas, breast, endometrial, or prostate cancer. Presence of GMPs was associated with markers of aggressive tumor behavior and significantly reduced survival or increased clinical recurrences in all four of the cancer types in univariate analysis. GMPs were related to increased microvessel density in prostate cancer only. In the case of melanomas, breast, and prostate cancers (but not endometrial cancers), GMPs were a significant prognostic factor in the final multivariate models (P all ≤ 0.02), and was a better predictor of outcome than was microvessel density. In conclusion, GMPs might indicate a more aggressive vascular phenotype associated with poor prognosis and could be a novel prognostic marker in human cancer.

Introduction

GMPs⁶ (or glomeruloid bodies) are focal proliferative buddings of endothelial cells resembling a renal glomerulus (1, 2). Classically, GMPs are one of the defining histological features of glioblastoma multiforme (3) and have been associated with increased aggressiveness in brain tumors (1, 4). Recently, Sundberg *et al.* (5) described a novel animal model for the development, stabilization, and regression of GMPs by local injection of an adenovirus vector directing VEGF expression. The aim of our present study was to evaluate the presence of GMPs in relation to MVD and patient prognosis in a well-defined series of human cancer, *i.e.*, cutaneous melanoma, breast cancer, endometrial cancer, and prostate cancer. To our knowledge, the clinical importance of this specific angiogenic phenotype has not been well studied in human cancers. Our data revealed a strong and significant prognostic impact of GMPs, indicating that this feature might be an important signature of a more aggressive vascular phenotype in several different tumor types.

Materials and Methods

For this study, four well-defined patient series were included. First, 202 consecutive vertical growth phase melanomas of the nodular type from Hordaland County, Norway (1981–1997), were examined (6); 188 had sufficient tissue left for additional studies. Information on patient survival, time, and cause of death was complete (median follow-up time for survivors: 88 months; range, 24–221; 72 melanoma deaths). Second, breast cancers included all of the patients (< 65 years at diagnosis) who reported themselves as being Ashkenazi Jewish by birth and were diagnosed with a first primary invasive breast cancer in 1980–1995 at the Sir Mortimer B. Davis-Jewish General Hospital. Of 292 consecutive cases, sufficient tumor tissue and follow-up data were present in 251 cases. Follow-up information was obtained by chart review (median follow-up time for survivors: 111 months; range, 1–249; 78 breast cancer deaths). Clinicopathologic details for a subset of these cases have been reported elsewhere (7). Third, of all 316 endometrial carcinoma patients occurring in Hordaland County, Norway, during 1981–1990 (8), a subgroup of 195 patients with available sections was studied. Information on survival, time, and cause of death was complete (median follow-up time for survivors: 102 months; range, 48–189; 43 cancer deaths). Fourth, a consecutive series of 104 men from Hordaland County, Norway, treated by radical prostatectomy for clinically localized prostate cancer during 1988–1994 was studied (9). Information on clinical locoregional recurrences or metastases was complete (median follow-up time for recurrence free survivors: 50 months; range, 20–128; 23 recurrences were observed).

Staining of endothelial cells by Factor-VIII (A-0082; Dako, Copenhagen, Denmark) was performed on formalin-fixed and paraffin-embedded archival material as published previously (6). GMPs were defined according to previous reports (2, 5, 10), with minor modifications. Presence of GMPs was recorded by the finding of focal glomerulus-like aggregates of closely associated and multilayered Factor-VIII positive endothelial cells. Countable GMPs or “bodies” consisted of 15–100 cells. Lumen formation was not necessary for the aggregates to be counted as GMPs. Tangentially sectioned vessels, or nonspecific Factor-VIII positivity in stromal components, were avoided. Tumors were sampled by examining one histological slide for the presence of GMPs, with selection of areas with highest tumor grade in the case of heterogeneity. Furthermore, GMPs were counted in the most active areas by $\times 100$ magnification in at least four consecutive fields (0.16 mm²/field), and reported as number/mm² in melanomas and endometrial cancers. The median number of GMPs was 0.21 and 0/mm² in melanomas and endometrial cancers, respectively, and these cases were grouped into two (negative/positive) by the 75 percentile. Counting was not performed in the cases of breast and prostate cancer, because these tumors had even fewer GMPs/mm²; these were grouped by absence (0) or presence (≥ 1) of GMPs per tumor. In addition, sections from 7 cases of melanoma and 3 breast cancers (1 slide/case) were examined by immunohistochemistry, including double staining using a mouse monoclonal antibody against α -SMA for the presence of pericytes and smooth muscle cells in GMPs and Factor-VIII as an endothelial cell marker. Finally, evaluation of MVD was performed as described previously (6, 8, 9, 11).

Associations between categorical variables were assessed by Pearson's χ^2 test. Continuous variables not following the normal distribution were compared between two or more groups using Mann-Whitney U or Kruskal-Wallis

Received 7/29/02; accepted 10/10/02.

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¹ Supported by Norwegian Cancer Society Grant D-94070 (to L. A. A.), Canadian Genetic Diseases Network, and United States Army Medical Research and Materiel Command DAMD17-98-1-8112 (to W. D. F.)

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⁶ The abbreviations used are: GMP, glomeruloid microvascular proliferation; VEGF, vascular endothelial growth factor; α -SMA, α smooth muscle actin; MVD, microvessel density.

H tests. Univariate analyses of time to death because of cancer or time to recurrence were performed using the product-limit procedure (Kaplan-Meier method) with the log-rank test. The influence of covariates on the patient survival function and recurrence-free survival function was analyzed by the proportional hazards method, including all of the variables with a $P \leq 0.15$ in univariate analyses, and tested by the likelihood ratio (lratio) test. Model assumptions were tested by log-minus-log plots, and significant variables were tested for interactions.

Results and Discussion

Mean number of GMPs in melanomas was 0.41/mm² (median 0.21/mm²), compared with a mean of 0.25/mm² in endometrial carcinomas; median values for sites other than melanomas were 0/mm². Forty-four melanomas (23%) and 44 endometrial carcinomas (23%) were classified as positive for GMPs. Correspondingly, 43 breast cancers (17%) and 14 cases of prostate cancer (13%) were GMP

positive (Fig. 1). Presence of GMPs was associated with increased MVD only in the series of prostate cancer; median MVD in GMP-negative cases was 122 vessels/mm² compared with 165 vessels/mm² in GMP-positive tumors (Mann Whitney *t* test; $P = 0.013$). As illustrated in Fig. 2, GMPs also contained cells positive for α -SMA, suggesting the presence of pericytes and smooth muscle cells. This positivity was most pronounced in “late-stage” GMPs (5) but tended to be weaker than in the more mature vessels at the invasive tumor front (Fig. 2B). In melanomas and endometrial carcinomas, data on immunohistochemical expression of VEGF in tumor cells was available (6, 12). However, no clear and simple relationship between the expression level of VEGF and GMPs was present (data not shown).

GMPs were related to several basic variables. In melanomas, GMPs were associated with tumors on extremities ($P = 0.012$), tumor ulceration ($P = 0.001$), and increasing thickness ($P = 0.007$). In

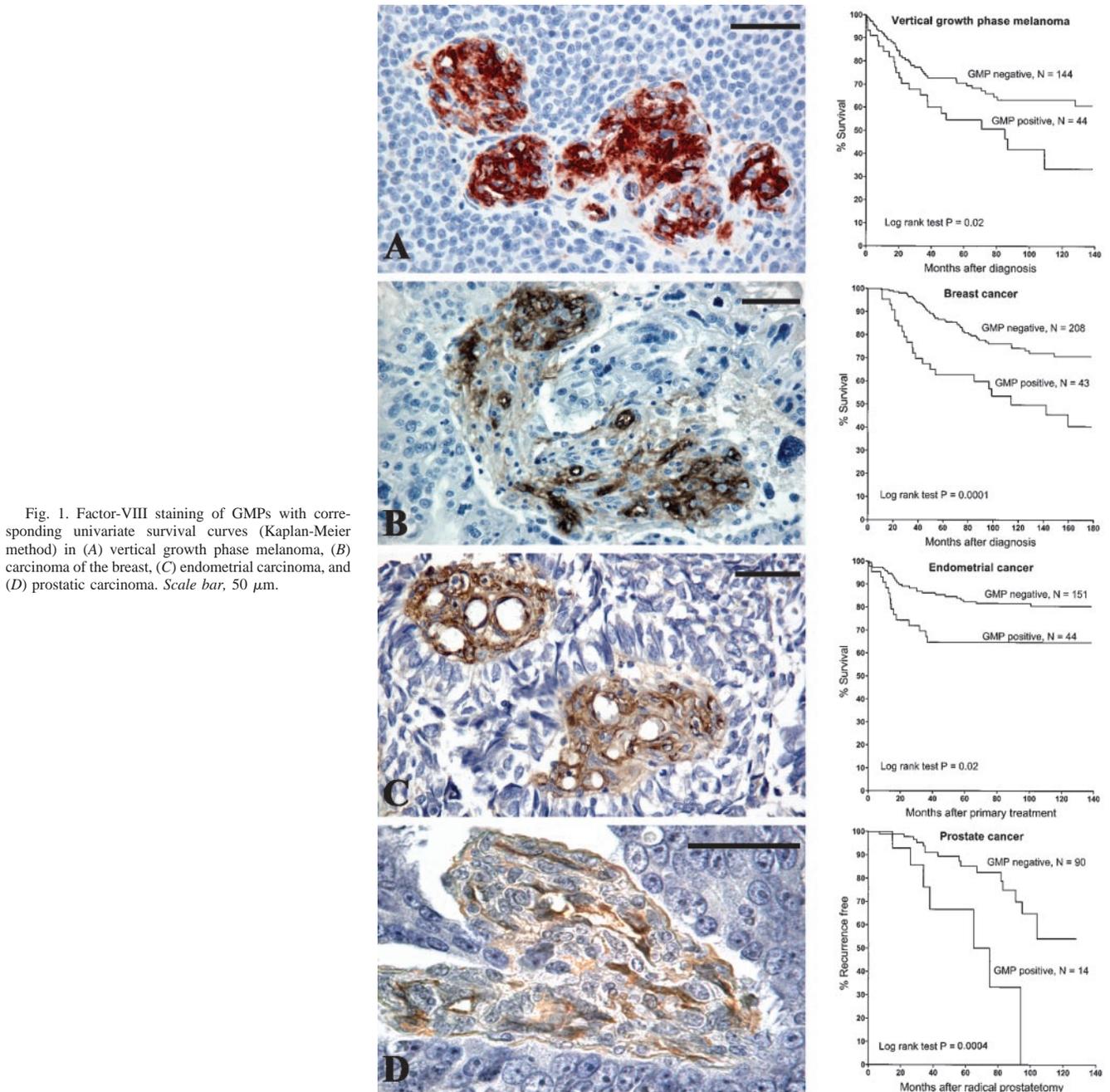


Fig. 2. The mouse monoclonal antibody (M 0851, clone 1A4I DAKO) raised against α -SMA was detected by the EnVision (K4004; DAKO; 1:200) detection system to illustrate the presence of pericytes/smooth muscle cells in GMPs. A, GMP in a melanoma case with cells positive for α -SMA in red (dark blue dots are erythrocytes). B, mature tumor vessels at the invasive front of the same case with strong α -SMA positivity. C, GMP in a breast cancer case with cells positive for α -SMA. D, simultaneous indirect double staining was performed using the mouse anti- α -SMA antibody and rabbit anti-Factor-VIII antibody (1:1000). α -SMA was detected by a biotinylated goat antimouse antibody (E 0433; DAKO) and alkaline phosphatase-conjugated StreptABComplex (K 0391; DAKO), and stained with fast blue. Factor-VIII was detected with EnVision and stained with 3-amino-9-ethylcarbazole (red). GMP in a breast carcinoma being positive for α -SMA and Factor-VIII. Scale bar, 50 μ m.

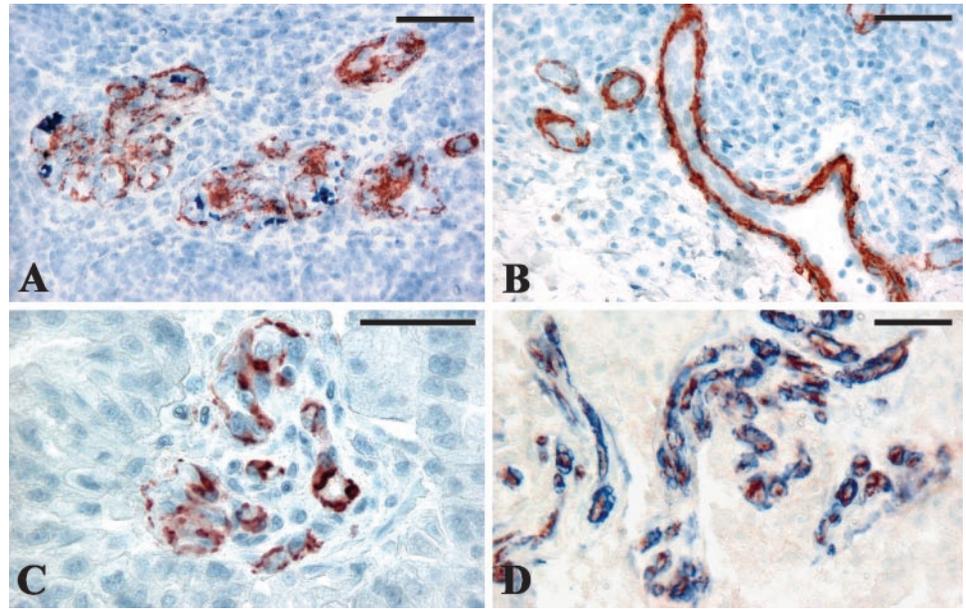


Table 1 Multivariate survival analysis: final models according to the proportional hazards method, for patients with melanoma, breast cancer, endometrial cancer, and prostate cancer, using death from cancer as end point (clinical recurrence in prostate cancer).

Only patients with information on all variables were included. Variables included in step 1 of the multivariate analysis: melanoma: level of invasion (Clark), tumor thickness, GMPs, vascular invasion, ulceration, anatomic site, MVD; breast cancer: tumor size, tumor type (ductal vs. others), nuclear grade, lymph node status, estrogen receptor status, progesterone receptor status, GMPs; endometrial cancer: age, FIGO-stage, histologic type, histologic grade, MVD, GMPs; prostate cancer: histologic grade (WHO), GMPs (other basic variables had $P > 0.15$ in univariate analysis). FIGO, Federation Internationale des Gynaecologues et Obstetristes.

Variables	Categories	n	HR ^a	P ^b
Melanoma				
Level of invasion (Clark)	II, III, IV	134	1	
	V	31	5.1	<0.0001
Anatomic site	Others	118	1	
	Trunk	47	4.6	<0.0001
MVD	Low ^c	109	1	
	High	56	2.2	0.007
GMPs	Negative	125	1	
	Positive	40	2.6	0.001
Breast cancer				
Tumor size	≤1.6 cm (median)	100	1	
	>1.6 cm (median)	118	2.1	0.003
Lymph nodes involved	0	119	1	
	1–3	58	1.1	
	>3	41	3.9	<0.0001
Nuclear grade	Low	56	1	
	Moderate	89	1.2	
	High	73	2.5	0.018
GMPs	Negative	180	1	
	Positive	38	2.4	0.003
Endometrial cancer				
Age at operation FIGO stage	Continuous variable	194	1.04	0.013
	I/II	158	1	
	III/IV	36	8.9	<0.0001
Histologic grade	Well	47	1	
	Moderate	105	2.0	
	Poor	42	4.7	0.003
MVD	Low ^d	148	1	
	High	46	2.3	0.011
GMPs	Negative	151	1	
	Positive	43	1.3	0.4
Prostate cancer				
Histologic grade (WHO)	Well/moderate	76	1	
	Poor	28	2.8	0.02
GMPs	Negative	90	1	
	Positive	14	3.5	0.015

^a Hazard ratio.

^b L ratio test.

^c 67 percentile.

^d 75 percentile.

breast cancers, GMPs were associated with ductal type ($P = 0.023$), high nuclear grade ($P < 0.001$), estrogen receptor negativity ($P < 0.001$), and Her-2/*neu* positivity ($P = 0.03$). In endometrial cancers, GMPs were associated with moderately/poorly differentiated tumors ($P = 0.015$) and Fédération Internationale des Gynaecologues et Obstetristes stage III/IV ($P = 0.026$). In prostate cancers, GMPs were associated with high preoperative serum-PSA ($P = 0.026$). GMPs were significantly associated with patient survival (melanoma, breast cancer, and endometrial cancer) and clinical recurrence (prostate cancer) by univariate analysis, as illustrated in Fig. 1. In multivariate analysis, all of the basic prognostic factors (see Refs. 6, 8, 9; Table 1) with a $P \leq 0.15$ were initially included. Table 1 shows the last step in multivariate analysis (backward stepwise selection procedure).

In the present study, we show that GMPs occur in 13–23% of four human tumor types, and this vascular signature appears to indicate a more aggressive tumor phenotype. Whether GMPs are related to an accelerated angiogenesis or represent a dysfunctional angiogenic response is not known and should be studied additionally. GMPs were associated with increased MVD in prostate cancer only, whereas no association was observed for the other tumors. In the case of melanoma, breast, and prostate cancers, GMPs were a significant predictor of outcome in the final multivariate models, and a better prognostic marker than was MVD. MVD, as described by Weidner *et al.* (11), is a widely used estimate of the angiogenic process in histological sections, showing prognostic impact in several tumor types, including breast cancer (11, 13), prostate cancer (9, 14), endometrial carcinoma (8), and melanoma (6). Recently, Hlatky *et al.* (15) pointed out the limitations of MVD and the need for improved angiogenic features. Our present findings indicate that evaluation of angiogenesis by GMPs might serve as a novel prognostic marker, indicating a more aggressive angiogenic phenotype.

Acknowledgments

We thank Gerd Lillian Hallseth, Nancy Hamel, Bendik Nordanger, Ann-Josée Paradis, and Nora Wong for excellent database and technical assistance.

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Cancer Res 2002;62:6808-6811.

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