PEA3 Is the Second Ets Family Transcription Factor Involved in Tumor Progression in Ovarian Carcinoma[1]

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ABSTRACT

Purpose: **The purpose of this study was to analyze the possible correlation between PEA3 mRNA expression and survival in advanced-stage ovarian carcinomas, studying two patient groups with extremely different disease outcome.**

Experimental Design: **Sections from 61 primary ovarian carcinomas and metastatic lesions from 36 patients diagnosed with advanced-stage ovarian carcinoma [International Federation of Gynecologists and Obstetricians (FIGO) stages III-IV] were evaluated for expression of PEA3 using mRNA *in situ* hybridization. Patients were divided into long-term ($n = 16$) and short-term ($n = 20$) survivors.**

Results: **The mean values for disease-free survival and overall survival were 119 and 137 months for long-term survivors, as compared with 4 and 22 months for short-term survivors, respectively. Expression of PEA3 mRNA was detected in carcinoma cells and stromal cells in 56 of 61 lesions (92%) and 54 of 61 lesions (89%), respectively. Intense stromal expression was detected only in the vicinity of grade 2–3 tumors ($P = 0.04$). PEA3 expression in stromal cells showed a significant association with matrix metalloproteinase 2 mRNA expression in carcinoma cells ($P = 0.022$). PEA3 expression in carcinoma cells showed an association with mRNA expression of the β_1 integrin subunit in the same compartment ($P = 0.039$). It was also associated with mRNA expression of β_1 integrin subunit ($P = 0.012$), basic fibroblast growth factor ($P = 0.036$), and the matrix metalloproteinase inducer EMMPRIN ($P = 0.038$) in stromal cells. PEA3 mRNA was detected more often in both carcinoma and stromal cells in tumors of short-term survivors ($P = 0.021$ for stromal cells). In univariate survival analysis, PEA3 expression in stromal cells correlated with both shorter disease-free survival ($P = 0.019$) and overall survival ($P = 0.029$), whereas tumor cell expression predicted poor overall survival ($P = 0.049$). PEA3 mRNA expression in stromal cells emerged as an independent predictor of poor outcome in multivariate survival analysis, in which all molecules previously studied in this patient cohort were included ($P = 0.015$).**

Conclusions: **To the best of our knowledge, this is the first evidence associating PEA3 mRNA expression and poor survival in human epithelial malignancy. PEA3 is thus a novel prognostic marker in advanced-stage ovarian carcinoma. The association between PEA3 mRNA expression and the expression of the β_1 integrin subunit, basic fibroblast growth factor, and EMMPRIN, first documented in our patient cohort, points to the central role of this transcription factor in tumor progression in ovarian carcinoma.**

INTRODUCTION

Local invasion and distant metastasis are the main factors responsible for cancer-related morbidity and mortality. Both processes are a function of the complex interactions between tumor cells and the surrounding stroma, which are mediated by the production of a wide variety of molecules, among which matrix-degrading proteases and angiogenic factors play a central role (1, 2). Activation and repression of synthesis of these molecules are mediated through the relay of extracellular signals, with the resulting activation of transcription factors.

The Ets family of transcription factors is divided into subfamilies (Ets-1 and -2, ERG, GABP, PEA3, ELK, ELF, and PU1) based mainly on the sequence and location of the Ets domain, an 84-amino acid sequence present in all members of the family. Ets proteins bind to DNA sequences with the core motif C/A-GCA-A/T (3). Ets transcription factors play a role in a variety of physiological and pathological processes, including embryogenesis, wound healing, and tumor progression (3–5). This is largely due to their ability to activate the transcription of proteases, including urokinase-type plasminogen activator (6) and MMPs[3] (7), as well as that of TIMPs (7) and β_3 integrin (8). The activation of proteolytic enzyme transcription is central to the metastatic process because of the role of these enzymes in both angiogenesis and tumor invasion.

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[3] The abbreviations used are: MMP, matrix metalloproteinase; ISH, *in situ* hybridization; bFGF, basic fibroblast growth factor; FIGO, International Federation of Gynecologists and Obstetricians; TIMP, tissue inhibitor of metalloproteinase.

Table 1 Tissue distribution of primary tumors and metastatic lesions according to tumor type

Tumor type	No. of patients	Ovary	Omentum	Peritoneum	Intestine	LN[a]	Other	Total no. of biopsies
Serous	28	24	9	6	3	3	2	47
Endometrioid	3	3	2	0	0	0	0	5
Mucinous	2	2	0	0	1	0	0	3
Undifferentiated	1	1	0	1	0	0	0	2
Clear cell	1	1	1	0	0	0	1	3
Neuroendocrine	1	1	0	0	0	0	0	1
Total	36	32	12	7	4	3	3	61

[a] LN, lymph node.

The PEA3 subfamily includes PEA3, ER81, and ERM (9–11). The human PEA3 homologue, E1AF, shares a 94% homology with PEA3 and has an identical Ets domain (9, 12). It binds to the enhancer elements of the adenovirus type 5 E1A gene (13) and has been shown to up-regulate MMP transcription (14). Among epithelial malignancies, breast cancer has been the most extensively studied. PEA3 overexpression was reported in primary and metastatic lesions of mouse mammary carcinoma (15), and expression of its dominant-negative form reduced tumor growth in this model (16). PEA3 mRNA expression showed an association with HER2/neu expression, but not with estrogen receptor status, nuclear grade, or S-phase fraction, in clinical specimens of breast carcinoma (17), and PEA3 protein expression predicted worse overall survival in this malignancy (18). Conversely, PEA3 expression suppressed HER2/neu expression in human breast and ovarian carcinoma (19) and mediated apoptosis in the SKBr-3 breast carcinoma cell line (20). In addition, PEA3 subfamily members were detected in benign breast epithelium and estrogen receptor-positive tumor cells, but not in their receptor-negative counterparts (21). PEA3 expression was found in the majority of both clinical specimens and cell lines of lung and oral carcinoma (22–24). Transfection with PEA3 resulted in enhanced motility and invasion in lung cancer cells (22), whereas transfection of oral carcinoma cells with an antisense sequence resulted in inhibition of invasion and MMP expression (23). As opposed to breast and lung tumors, PEA3 was reported to be absent in prostate carcinoma (25). Although reported in ovarian carcinoma cell lines (26), PEA3 expression in patient material and its role in the clinical course of ovarian carcinoma are unknown to date.

Ovarian cancer is the sixth most common cancer and the sixth most frequent cause of cancer death in women (4.4% of cases and 4.5% of deaths), and it is the leading cause of death from gynecologic cancer in women in industrialized countries (27). Despite the inclusion of new chemotherapeutic regimens, the mortality rate from ovarian carcinoma has remained essentially unchanged, mainly due to the late clinical presentation of this tumor. However, it is crucial to attempt to further characterize different prognostic groups of patients within each given stage by the use of molecular markers. Studying the same patient cohort in a series of investigations of metastasis-related gene expression, we have been able to highlight the role of mRNA expression of Ets-1 (4), MMPs and their inhibitor TIMP-2 (28), and the α_v integrin subunit (29) as a predictive factor for disease aggressiveness in ovarian carcinoma. Conversely, mRNA expression of the MMP inducer EMMPRIN[4] and the angiogenic factors bFGF, interleukin 8, and vascular endothelial growth factor (30) did not influence the prognosis of patients in this cohort. The present study evaluates the expression and prognostic value of PEA3 mRNA in ovarian carcinoma and investigates the potential association between PEA3 expression and that of the previously studied molecules.

PATIENTS AND METHODS

Patients. The study population consisted of 36 patients, diagnosed with advanced-stage (FIGO III-IV) epithelial carcinoma of the ovary in the Division of Gynecologic Oncology at the Sheba Medical Center in the period between 1977 and 1996. The patient cohort was retrospectively selected for good and poor outcomes. The study cohort was thus divided into two groups, consisting of 16 and 20 patients, defined as long-term and short-term survivors, respectively, using a double cutoff of 36 months for disease-free survival and 60 months for overall survival. Clinicopathological parameters were the sole inclusion criteria, with the object of creating two groups with comparable profiles regarding histological grade, FIGO stage, age, and residual disease but markedly different outcome. In-patient and out-patient charts were available for review for all patients. No patients were lost to follow-up. All patients underwent surgery, followed by standard chemotherapy protocols. Until 1995, patients received adjuvant chemotherapy, including cisplatinum and cyclophosphamide. Since 1995, paclitaxel has replaced cyclophosphamide.

Tumors. Sixty-one formalin-fixed, paraffin-embedded blocks from the archives of the Department of Pathology at the Sheba Medical Center were included in the study. These consisted of 32 primary ovarian tumors and 29 metastatic lesions from 36 patients with advanced ovarian carcinoma. The distribution of the studied material according to biopsy site is shown in Table 1. Sections from all tumors were reviewed by two observers (B. D. and J. K.) in consensus sessions to confirm the diagnosis, histological type, and tumor grade (I-III, corresponding to well, moderately, and poorly differentiated). Established criteria were used for the microscopic diagnosis and tumor classification (31). Tumor staging was established according to FIGO criteria.

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Table 2 Survival data for long-term and short-term survivors

Patient group	Mean disease-free survival (mo)	Mean overall survival (mo)	DOD[a]	AWD	NED
Long-term survivors ($n = 16$)	119	137	2	3	11
Short-term survivors ($n = 20$)	4	22	20	0	0

[a] DOD, dead of disease; AWD, alive with disease; NED, no evidence of disease.

Oligonucleotide Probe. Specific antisense oligonucleotide DNA probe for the mRNA transcript of PEA3 was obtained from Research Genetics (Huntsville, AL). The probe sequence (5′ to 3′) was as follows (19): 5′-TGA-ATT-ATG-AGA-AGC-TGA-GCC-G-3′.

The specificity of the probe was verified using a sense probe (Research Genetics). The sense probe sequence was as follows: 5′-CGG-CTC-AGC-TTC-TCA-TAA-TTC-A-3′.

A poly d(T)20 oligonucleotide probe (Research Genetics) was used to verify the integrity and lack of degradation of mRNA in each sample. cDNA probes were hyperbiotinylated. The stock dilution was diluted with probe diluent (Research Genetics) immediately before use.

mRNA ISH. Tissue sections (4 μm thick) of formalin-fixed, paraffin-embedded specimens were mounted on ProbeOn Plus slides (Fisher Scientific, Pittsburgh, PA). Sectioning was performed in RNAase-free water. mRNA ISH was carried out by using the microprobe manual staining system (Fisher Scientific; Ref. 32). Hybridization of the probes was carried out as described previously (28). A positive enzymatic reaction in this assay stained dark blue. Known positive controls were used in each hybridization reaction. These consisted of two cases for which positive hybridization was reproducible in pilot studies. Controls for endogenous alkaline phosphatase included treatment of the sample in the absence of the probe and use of chromogen alone.

Evaluation of mRNA ISH Results. Staining was scored in carcinoma and stromal cells. Staining extent was scored as 0, 1, or 2, using a cutoff of 20%. Staining of ≤20% of tumor/stromal cells was scored as focal (1), whereas staining of >20% of cells was interpreted as diffuse (2). Staining intensity was scored as absent (0), weak/moderate (1), or intense (2).

Statistical Analysis. Staining values in tumor cells and stromal cells were evaluated statistically, applying the SPSS PC package (Version 10.1; SPSS, Chicago, IL). Probability of <0.05 was considered statistically significant. Analyses of the association between mRNA ISH results and biopsy site, patient group, tumor grade, and previously studied metastasis-associated molecules were executed using the two-sided χ^2 test. Univariate survival analyses were executed using the Kaplan-Meier method and log-rank test. Both staining extent and intensity were analyzed. Multivariate analyses of survival were performed using the Cox regression model. The parameters included all previously studied markers that showed significance in univariate analysis of this cohort: mRNA expression of MMP-2, MMP-9, MT1-MMP, TIMP-2, Ets-1, and α_v integrin, as well as expression of the carbohydrate antigen sialyl Lewis^x (33) and the protein expression of the adhesion molecule γ-catenin (34). Clinical parameters (patient age, tumor type, grade of differentiation, and disease stage) were not included because their prognostic role was nullified by the choice of patients for this study.

RESULTS

Patients

Patient age ranged from 30 to 84 years, with a mean age of 56.2 and 57.6 years in the long-term and short-term survivor group, respectively. Thirty patients were diagnosed with stage III tumors, and six patients were diagnosed with stage IV tumors. These were equally represented in the two patient groups. Thus, the long-term survivor group included 13 patients diagnosed with stage III tumors and 3 patients with stage IV tumors, whereas the short-term survivor group included 17 patients diagnosed with stage III tumors and 3 patients diagnosed with stage IV tumors, respectively. Follow-up period ranged from 8 to 224 months (mean, 69 months). Mean disease-free survival and overall survival data, as well as disease status, are presented in Table 2.

Tumors

The primary tumor diameter was comparable for both patient groups, ranging from 2.2 to 15 cm in the long-term survivor group and from 3 to 16 cm in the short-term survivor group. The distribution of tumors according to histological type is shown in Table 1. Tumor differentiation was as follows: 6 grade I tumors; 3 grade II tumors; and 27 grade III tumors. The fraction of poorly differentiated (grade III) tumors was comparable for both groups (11 of 16 and 16 of 20 tumors).

mRNA ISH

A positive signal using a poly d(T) probe was detected in all cases (Fig. 1*A*). Sections hybridized with the sense probe were uniformly negative, as were controls hybridized without probe (Fig. 1*B*). Expression of PEA3 mRNA was detected in carcinoma cells and peritumoral stromal cells in 56 of 61 lesions (92%) and 54 of 61 lesions (89%), respectively (Fig. 1, *C*–*E*). Labeling of tumor cells was intense in 5 specimens, whereas in 14 specimens, similar findings were seen in the stromal compartment. Labeling extent in tumor and stromal cells was categorized as diffuse in 45 specimens each.

Statistical Analysis

Clinicopathological Parameters. Intense labeling for PEA3 mRNA was detected more often in both carcinoma and stromal cells in the tumors of short-term survivors ($P = 0.021$ for stromal cells). Labeling extent in stromal cells showed the same trend, although it was not significant (Table 3). Intense stromal expression was detected only in the vicinity of grade 2–3 tumors ($P = 0.04$; Table 4). Stromal

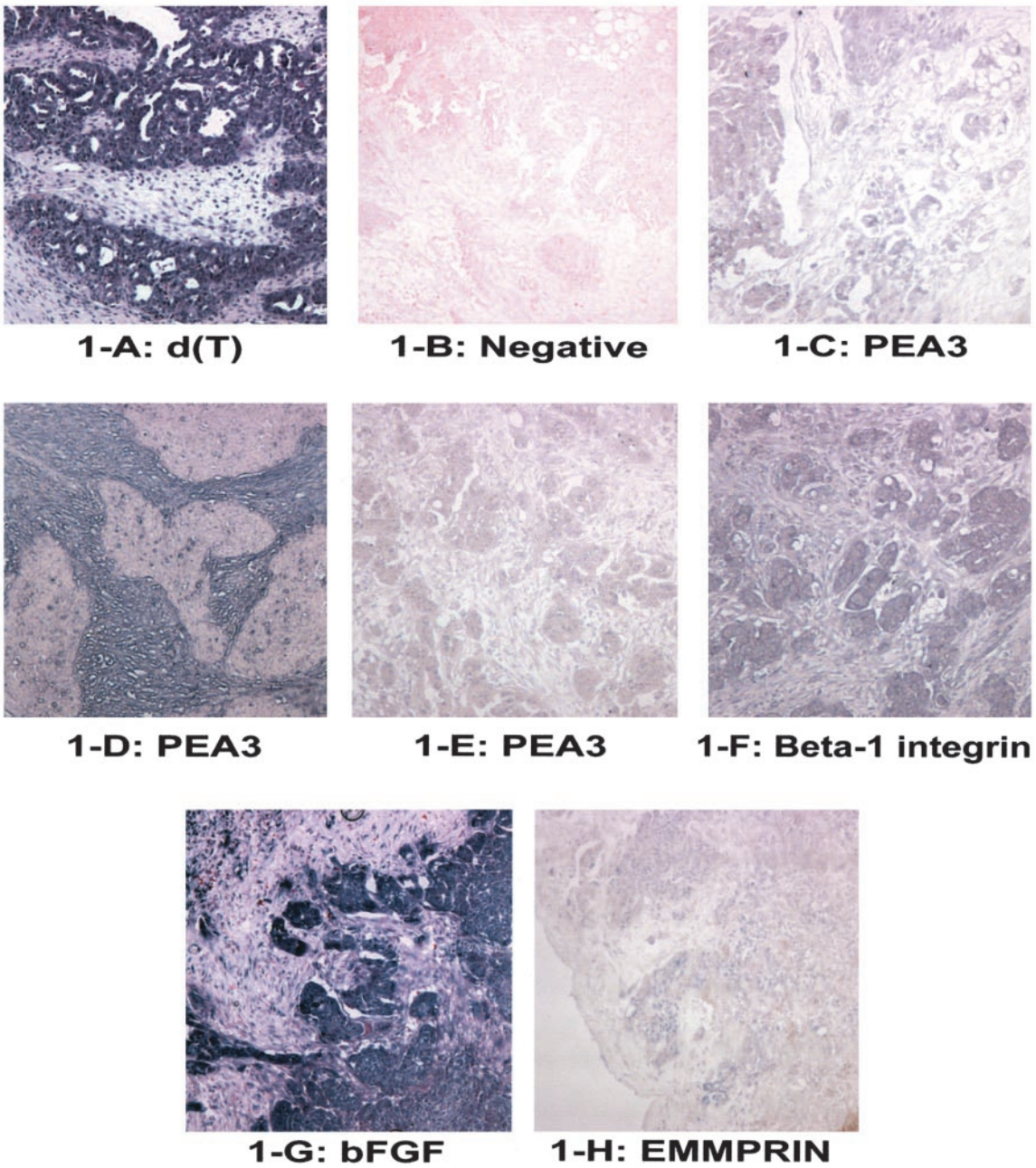

Fig. 1 PEA3 mRNA expression in advanced-stage ovarian carcinoma. *A*, d(T) control for mRNA integrity in a primary ovarian serous carcinoma. All cells are labeled (nitroblue tetrazolium-5-bromo-4-chloro-3-indolyl phosphate staining). *B*, an intestinal metastasis hybridized with a sense probe showing no labeling. Cells are counterstained with nuclear fast red. *C* shows weak, diffuse PEA3 expression in both the tumor and stromal cell compartments in a peritoneal metastasis from a short-term survivor diagnosed with FIGO stage IIIc serous carcinoma. *D*, diffuse intense expression (*dark blue*) of PEA3 mRNA in stromal cells of an omental metastasis in a FIGO stage IV serous carcinoma of a short-term survivor. Weak staining of tumor cells is also seen. *E* and *F* demonstrate coexpression of PEA3 and β_1 integrin subunit mRNA in an omental metastasis from a long-term survivor. Both tumor and stromal cells are positive. *G* and *H* demonstrate coexpression of bFGF and EMMPRIN in the tumor shown in *C*. Stromal and tumor cells are labeled.

expression was also more diffuse in tumors from older patients ($P = 0.066$; data not shown). PEA3 expression was comparable in primary and metastatic lesions and showed no association with FIGO stage (data not shown).

Previously Studied Metastasis-associated Molecules. Intense PEA3 expression in stromal cells showed a significant association with MMP-2 mRNA expression in carcinoma cells ($P = 0.022$; Table 5). Intense PEA3 mRNA expression in

Table 3 The distribution of PEA3 mRNA expression in tumors of long-term ($n = 27$) and short-term ($n = 34$) survivors[a]

A. Intensity

Compartment	Absent	Weak/moderate	Intense
Long-term survivors			
Tumor cells	2 (7%)	25 (93%)	0 (0%)
Stromal cells	5 (19%)	20 (74%)	2 (7%)
Short-term survivors			
Tumor cells	3 (9%)	26 (76%)	5 (15%)
Stromal cells	2 (6%)	20 (59%)	12 (35%)

B. Extent

Compartment	0%	1–20%	21–100%
Long-term survivors			
Tumor cells	2 (7%)	5 (19%)	20 (74%)
Stromal cells	5 (19%)	3 (11%)	19 (70%)
Short-term survivors			
Tumor cells	3 (9%)	6 (18%)	25 (73%)
Stromal cells	2 (6%)	6 (18%)	26 (76%)

[a] $P = 0.021$ for intensity in stromal cells.

Table 4 The association between labeling intensity for PEA3 mRNA in stromal cells and histological grade

	PEA3				
Grade	Absent	Weak	Intense	Total	
I	1	9	0	10	$P = 0.04$
II	0	2	4	6	
III	6	29	10	45	
Total	7	40	14	61	

Table 5 The association between labeling intensity for PEA3 in stromal cells and MMP-2 mRNA in carcinoma cells (56 specimens)

	PEA3				
MMP-2	Absent	Weak	Intense	Total	
Absent	4	5	12	21	$P = 0.022$
Weak	0	5	20	25	
Intense	1	1	8	10	
Total	5	11	40	56	

carcinoma cells showed an association with mRNA expression of β_1 integrin in the same compartment ($P = 0.039$; Table 6; Fig. 1*F*). It also showed association with intense mRNA expression of the β_1 integrin subunit ($P = 0.012$) and bFGF ($P = 0.036$; Fig. 1*G*) and with diffuse labeling for EMMPRIN ($P = 0.038$) in stromal cells (Table 7; Fig. 1*H*).

Survival Analysis. In univariate survival analysis, intense stromal PEA3 expression predicted poor disease-free survival ($P = 0.019$) and overall survival ($P = 0.029$; Fig. 2, *A* and *B*). Intense expression in tumor cells similarly predicted poor overall survival ($P = 0.049$; Fig. 2*C*). PEA3 mRNA expression in stromal cells emerged as an independent predictor of poor outcome in multivariate survival analysis, in which all molecules previously studied in this patient cohort were included ($P = 0.015$), together with TIMP-2 mRNA expression in stromal cells ($P = 0.001$) and the expression of MMP-9 ($P = 0.046$), sialyl Lewis^x ($P = 0.049$), and γ-catenin ($P = 0.013$) in tumor cells.

DISCUSSION

Ovarian carcinoma is a highly metastatic tumor, with a unique dissemination pattern characterized by multifocal spread in the peritoneal cavity. The role of proteolytic enzymes in ovarian carcinoma has been studied extensively, and we have recently shown that although the expression of MMP-1 and MMP-2 predominates in primary and metastatic sites (35), other MMPs (MMP-9 and MT1-MMP) and TIMP-2 mRNA also identify tumors associated with a rapidly fatal course (28). Still, until recently, no data were available regarding the regulation of metastasis-associated molecules at the transcriptional level in ovarian carcinoma. Our initial studies of Ets transcription factor expression in advanced-stage ovarian carcinoma demonstrated Ets-1 mRNA expression in 30–40% of tumors in both solid lesions and effusions (4, 5). The present study attempted to evaluate the expression of PEA3, another member of this family with some overlapping promoter sites on MMP genes.

Early studies analyzing various benign and cancerous tissues have localized PEA3 mRNA and protein to carcinoma cells, whereas no expression was seen in benign tissue (17–18, 22, 25). PEA3 mRNA expression has been observed in >50% of breast, lung, and oral carcinomas (17, 22, 24). In an additional report, 47% of tumors expressed PEA3 protein, but expression below 10% was scored as negative (18). As opposed to early studies of Ets-1, stromal cell expression has not been documented. We found PEA3 expression in both tumor and stromal cells in the majority of the studied specimens, at both primary and metastatic sites. Furthermore, intense labeling was more frequently seen in the stromal compartment. The prominent expression in stromal cells is in agreement with the cellular localization of MMP-2, MMP-9, and TIMP-2 (28), as well as that of angiogenic genes (30), in this patient cohort. The central contribution of peritumoral fibroblasts to tumor progression has recently been reviewed (36). Our data provide additional evidence supporting the dual (tumor and stromal cell) origin of metastasis-associated molecules in ovarian carcinomas.

The presence of PEA3 sites in MMP promoters and its role in activation of proteolytic enzyme expression are well established (6, 7, 14, 23). Furthermore, induction of collagenase transcription after stimulation by bFGF and integrins has been demonstrated in experimental models (37–39). Coexpression of PEA3 and the angiogenic molecule interleukin 8 was shown in the HepG2 hepatoma cell line, as well as in three clinical specimens (40). However, no data are available regarding the association between PEA3 expression and that of integrins, proteolytic enzymes, or other angiogenic genes in clinical specimens of human tumors. We found a significant association between mRNA expression of PEA3 and that of MMP-2, bFGF, and the β_1 integrin subunit in our patient cohort. Coexpression of EMMPRIN, a member of the immunoglobulin superfamily involved in MMP-1, MMP-2, and MMP-3 activation, with PEA3 was also seen. This finding provides the first evidence of a possible association between these molecules *in vivo*. Furthermore, these associations highlight the role of tumor-stromal collaboration in ovarian carcinoma.

Table 6 The association between labeling intensity for PEA3 and β_1 integrin subunit mRNA in carcinoma cells (50 specimens)

	PEA3				
β_1 integrin	Absent	Weak	Intense	Total	
Absent	2	27	0	29	$P = 0.039$
Weak	0	11	4	15	
Intense	1	4	1	6	
Total	3	42	5	50	

Table 7 The association between labeling intensity for PEA3 in carcinoma cells and labeling intensity for bFGF (61 specimens) and β_1 integrin subunit (50 specimens) mRNA in stromal cells

	PEA3 (tumor cells)				
	Absent	Weak	Intense	Total	
bFGF (stromal cells)					
Absent	0	10	0	10	$P = 0.036$
Weak	4	17	0	21	
Intense	1	24	5	30	
Total	5	51	5	61	
β_1 integrin (stromal cells)					
Absent	2	31	1	34	$P = 0.012$
Weak	0	9	4	13	
Intense	1	2	0	3	
Total	3	42	5	50	

Establishing the relevance and predictive role of molecular markers requires evaluation of well-defined patient cohorts. Our study evaluated two groups of patients diagnosed with advanced-stage ovarian carcinoma with a markedly different disease outcome, with a follow-up period of up to 20 years. Established prognostic factors, such as age, stage, grade, and tumor type, were all controlled by patient selection criteria in the design of the study. This selection was meant to facilitate the study of potential prognostic markers. Results recently published using this patient cohort identified Ets-1 (4); MMP-2, MMP-9, MT1-MMP, and TIMP-2 (28); the α_v integrin subunit (29); sialyl Lewis^x antigen (33); the adhesion molecule γ-catenin (34); and topoisomerase II (41) as prognostic markers in advanced-stage ovarian carcinoma.

The present study evaluated the role of PEA3 as a prognosticator in this cohort. Prognostic studies of PEA3 in carcinomas are limited to two studies to date. Hida *et al.* (24) reported more frequent expression of PEA3 in oral carcinomas ($n = 27$) showing invasive growth or lymph node metastasis. Evaluating 89 patients diagnosed with breast carcinoma, Kinoshita *et al.* (18) found protein expression of PEA3 in tumor cells to be a predictor of poor overall survival at 6 years. In the present study, intense expression of PEA3 mRNA in both tumor and stromal cells correlated with poor overall survival. Stromal cell labeling also predicted poor disease-free survival. Furthermore, PEA3 expression in stromal cells retained its prognostic power in multivariate analysis, providing the first evidence of an independent predictive role for this molecule in human malignancy. Interestingly, two of the most powerful markers in Cox analysis (TIMP-2 and PEA3) were localized to stromal cells.

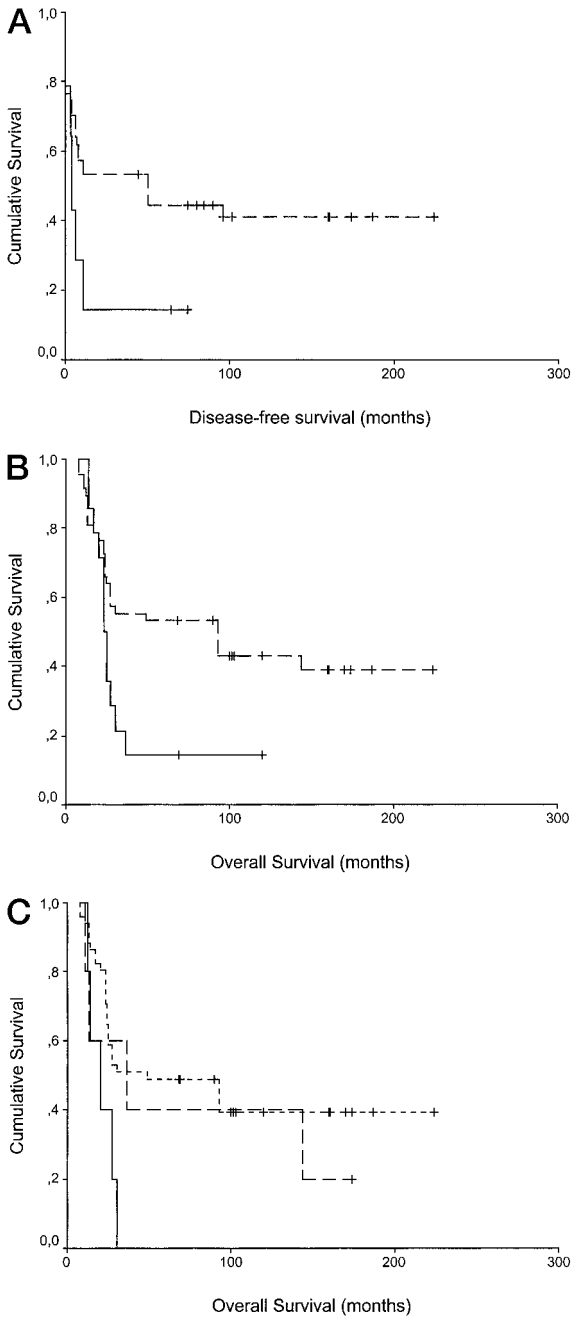

Fig. 2 Kaplan-Meier survival curves showing the correlation between PEA3 mRNA expression and disease outcome. *A*, the association between intense stromal cell PEA3 expression and disease-free survival. Patients with intensely labeled specimens ($n = 14$; *solid line*) had a mean disease-free survival of 14 months compared with 101 months for patients with weakly expressing or negative tumors ($n = 47$; *dashed line*; $P = 0.019$). Segregation of cases according to the three-tier system presented in "Results" showed comparable results ($P = 0.048$; data not shown). *B*, the association between intense stromal cell PEA3 expression and overall survival. Patients with intensely labeled specimens ($n = 14$; *solid line*) had a mean overall survival of 37 months, compared with 112 months for patients with weakly expressing or negative tumors ($n = 47$; *dashed line*; $P = 0.029$). *C*, the association between intense PEA3 expression in tumor cells and overall survival. Intense, weak, and negative expression (*solid*, *long-dashed*, and *short-dashed lines*, respectively) was associated with mean overall survival of 21, 108, and 76 months, respectively ($P = 0.049$).

This finding provides decisive evidence in attributing a central biological role to stromal cells in ovarian carcinoma. Parenthetically, advanced patient age and high histological grade are two of the established clinical prognostic markers in ovarian carcinoma (42). Although the predictive power of these parameters was nullified by the choice of patients for the long-term and short-term survivor groups, they did show an association with PEA3 expression.

Two tumors from short-term survivors showed no PEA3-positive cells in the tumor stroma, whereas intense expression was detected in stromal cells in two lesions from long-term survivors. Closer evaluation of these four cases revealed no unexpected data regarding clinical parameters. All four patients were diagnosed with FIGO stage III disease, had grade 3 tumors, and were about 60 years old. Similarly, the two short-term survivors with PEA3-negative tumors had an overall survival of 11 and 13 months, whereas the two long-term survivors with PEA3-positive tumors survived for 69 and 120 months, well in agreement with other patients' data. MMP-2, MMP-9, MT1-MMP, and TIMP-2 mRNA expression was variable. However, of interest is the fact that these two PEA3-negative cases were also Ets-1 negative in our earlier report, whereas the two PEA3-positive tumors from long-term survivors were also Ets-1 positive. This suggests induction of MMP synthesis by other transcription factors in short-term survivors with Ets-1- and PEA3-negative tumors and the presence of other, possibly growth-suppressive targets for PEA3 and Ets-1 regulation in few tumors from long-term survivors.

Transcriptional control of metastasis-associated genes is one of the critical parameters affecting tumor progression in malignant diseases. Our recent studies of Ets-1 and the present study of PEA3 indicate a possible regulatory pattern of these genes. In view of the central role of MMP-2, bFGF, EMMPRIN, and integrins in tumor invasion and angiogenesis, their coexpression and colocalization with PEA3 offers a possible explanation about the pathways leading to PEA3-mediated activation of metastasis-associated gene expression.

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