American Journal of Pathology, Vol. 137, No. 6, December 1990

Rapid Communication

Vimentin Expression Appears to Be Associated with Poor Prognosis in Node-negative Ductal NOS Breast Carcinomas

Wenancjusz Domagala,*‡ Jerzy Lasota,† Andrzej Dukowicz,† Maciej Markiewski,* George Striker,‖ Klaus Weber,‡ and Mary Osborn‡

From the Department of Tumor Pathology, Medical Academy, Szczecin, and the Department of Oncology,† Medical Academy, Lodz, Poland; and the Departments of Biochemistry‡ and Molecular Biology,‖ Max Planck Institute for Biophysical Chemistry, Goettingen, Federal Republic of Germany.*

Vimentin expression in tumors from 83 node-negative and 112 node-positive patients with infiltrative ductal not otherwise specified (NOS) breast carcinomas has been compared with 5-year survival. For node-negative, but not for node-positive patients, there was a significant inverse relation between vimentin expression and survival. Five-year survival of node-negative patients with vimentin-positive tumors was significantly worse compared with vimentin-negative tumors ($P < 0.0001$). In the node-negative group, only 36% of patients with vimentin-positive tumors but 82% of patients with vimentin-negative tumors survived 5 years. Tumors of all eight node-negative patients with ductal NOS cancer who died in the first 27 months expressed vimentin. Multivariate analysis of the node-negative group showed a strong correlation of vimentin expression and overall survival, but weak and not significant correlation between histologic grade or size and overall survival at 5 years. Thus vimentin expression seems to be a strong indicator of poor prognosis in node-negative ductal NOS breast carcinomas. (Am J Pathol 1990, 137:1299–1304)

Recently we showed that vimentin is preferentially expressed in infiltrative ductal not otherwise specified (NOS) breast carcinomas with low estrogen receptor and high Ki-67 growth fraction.[1] A positive correlation between vimentin and estrogen receptor-negative, epidermal growth factor receptor-positive human breast carcinomas has been documented.[2] In addition, preferential expression of vimentin in high-grade infiltrative ductal NOS breast carcinomas has been reported.[3–5] Thus vimentin expression in infiltrative ductal NOS breast cancer seems to be strongly associated with several poor prognostic indicators.[6–10] Therefore prognostic significance of vimentin expression in infiltrative ductal NOS breast carcinomas has been suggested[1,4,5] but not proved. To test this hypothesis, vimentin expression in breast cancer biopsy tissues that were formalin fixed and paraffin embedded is compared with survival curves for a 5-year period.

Materials and Methods

Specimens

Formalin-fixed and paraffin-embedded biopsies from 195 unselected mastectomy specimens from patients with primary infiltrative ductal NOS breast cancer and for whom survival data was available were examined. These were retrieved from the files of the Department of Oncology, Medical Academy of Lodz, Poland, where the patients had been examined between 1980 and 1985. On pathologic examination, 83 patients had negative lymph nodes and 112 had positive lymph nodes, with 1 to 20 nodes involved. Of 112 with positive lymph nodes, 25 had 1, 16 had 2, 14 had 3, 8 had 4, and 49 had 5 or more lymph nodes involved. All cases were reviewed histologically and representative sections were selected for immunocyto-

Supported in part by a grant from Mildred Scheel Stiftung of the Deutsche Krebshilfe to Mary Osborn.

Accepted for publication October 2, 1990.

Address reprint requests to: Dr. Mary Osborn, Max Planck Institute for Biophysical Chemistry, D-3400 Goettingen, Federal Republic of Germany.

chemical examination. The histologic classification of infiltrative ductal NOS carcinoma was performed according to published criteria.[11] Clinical information about the patients, including survival data for a period of 5 years, was obtained from hospital records.

Immunohistochemistry

The indirect immunoperoxidase method was used to determine positivity for keratin with the KL1 broad specificity mouse monoclonal antibody[12] (Dianova, Hamburg, FRG) and for vimentin with the mouse monoclonal V9 antibody[13] (for further details see Domagala et al[5]).

Statistical Analysis

To test the significance of the Kaplan–Meier survival plots, Chi-square values were calculated by log-rank statistics[14] (generalized Savage test). Because there were indications that the various parameters were noncumulative, multivariate analysis was performed by calculation of the coefficients of correlation in certain subgroups. Significance of correlation was determined by the *t* statistic.

Results

Vimentin and Keratin Expression

Table 1 presents data on the vimentin and keratin content of the 195 infiltrative ductal NOS breast carcinomas included in this study. Approximately 90% of these cases were included in our previous study of vimentin expression and histologic type.[5] Eighty-three cases were node negative, while in 112 cases metastases were found in one or more axillary lymph nodes. All were positive with the broad specificity keratin antibody (KL1), although not all tumor cells in every specimen were keratin positive (more than 75% in most cases).

Tumors were considered positive for vimentin when there was distinct brown cytoplasmic staining. Positive staining in fibroblasts, endothelial cells, lymphocytes, and macrophages and negative staining of epithelial cells in non-neoplastic tubules served as 'built-in' positive and negative controls, respectively. The percentages of vimentin-positive tumor cells were estimated semiquantitatively. As in previous studies,[1,4] tumors were considered positive when vimentin expression was detected in more than 10% of tumor cells. Thus the eight cases in which very small numbers of tumor cells (7 cases, less than 1%; and 1 case, less than 5%) were vimentin positive are not included in the vimentin-positive category in the tables. Of these eight cases, five were node negative and three were node positive. As shown in Domagala et al,[5] in the majority of vimentin-positive cases, more than 50% of the tumor cells stained for vimentin.

Vimentin and Survival

Tumor cells in 17% (34 of 195) of the cases expressed vimentin. Among node-negative patients, vimentin was present in 27% (22 of 83) of the cases (Table 1). In this group the survival curves of patients with vimentin-positive tumors and those with vimentin-negative tumors were significantly different ($P < 0.0001$; Figure 1A). All node-negative patients who died of cancer within the first 27 months were vimentin positive. Vimentin expression was found in 14% (8 of 58) of tumors of patients who survived 5 years while it was expressed in 56% (14 of 25) of tumors of those who died of the disease within 5 years (Table 2). On the other hand, among vimentin-positive cases, only 36% (8 of 22) survived 5 years, while 82% (50 of 61) 5-year survivors were found among vimentin-negative cases.

The survival curves of node-positive patients did not differ significantly with respect to vimentin expression (Figure 1B).

Histologic Grade and Survival

The difference in the effect of histologic grade on survival of the node-negative and node-positive groups is shown by the survival curves in Figures 2A and 2B. In these plots

Table 1. *Vimentin Expression and Histologic Grade in Node-negative and Positive Infiltrative Ductal NOS Breast Carcinomas*

		Vimentin*		Histologic grade			
Axillary lymph nodes	No. of cases	+ (%)	−	I	II	II/III	III
Negative	83	22 (27)	61	5	43	2	33
Positive	112	12 (11)	100	3	50	1	58
Total	195	34 (17)	161	8	93	3	91

* To be counted as vimentin positive, 10% or more of tumor cells had to express vimentin (see text).

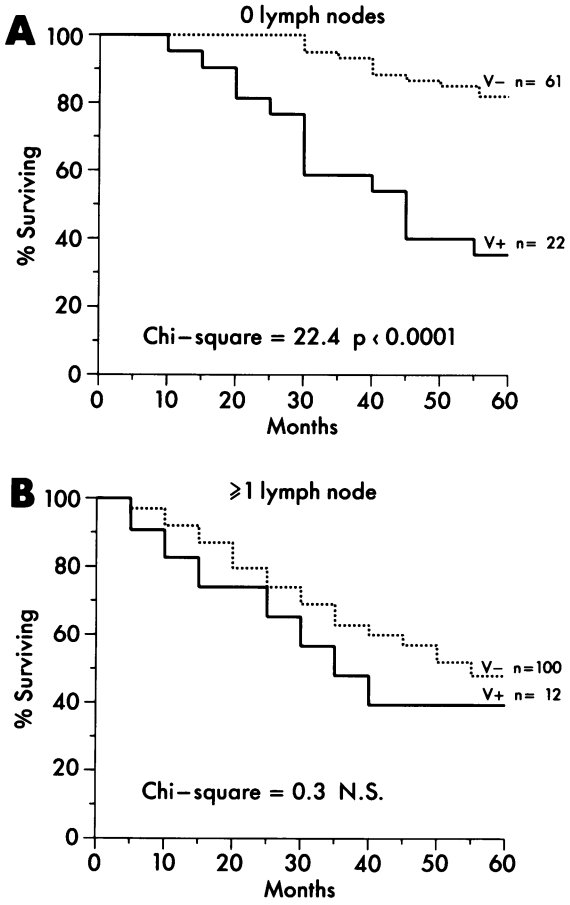

Figure 1. *Effect of vimentin content on survival curves of patients with infiltrating ductal NOS breast carcinoma.* **A**: *Node-negative cases.* **B**: *Cases with involvement of one or more axillary lymph nodes. V+ vimentin positive, V- vimentin negative.*

survival of patients with histologic grades I, II, and II/III is compared with that of histologic grade III. Comparison of Figures 1A and 2A for the node-negative group and of Figures 1B and 2B for the node-positive group shows that vimentin is the better prognostic parameter in the node-negative group, while tumor grade is the better parameter in the node-positive group.

Multivariate Analysis

The tables of cross-correlations between the various factors shown in Table 3 show considerable differentiation after segregation by lymph node status. When the total patient group is considered, grade and size correlate with survival (both 0.27) and there is a weaker but still significant correlation between vimentin expression and survival (0.19). In the node-positive patient group there is still a significant correlation of grade with survival (0.30), and of tumor size with survival (0.29), but there is no significant correlation between vimentin and survival (0.10). However the node-negative group shows a strongly significant correlation of vimentin with survival (0.51). In contrast, in this group tumor grade no longer correlates significantly with survival (0.17). Therefore grading appears to be the better prognostic factor in the node-positive group and vimentin is the better parameter in the node-negative group. The two factors are not cumulative.

Table 2. *Vimentin Expression and Survival in 82 Node-negative Infiltrative Ductal NOS Breast Carcinomas*

Time (years)	Dead		Alive	
	No.	Vim+ (%)	No.	Vim+ (%)
2	4	4/4 (100%)	79	18/79 (23%)
3	14	9/14 (64%)	69	13/69 (19%)
4	22	13/22 (59%)	61	9/61 (15%)
5	25	14/25 (56%)	58	8/58 (14%)

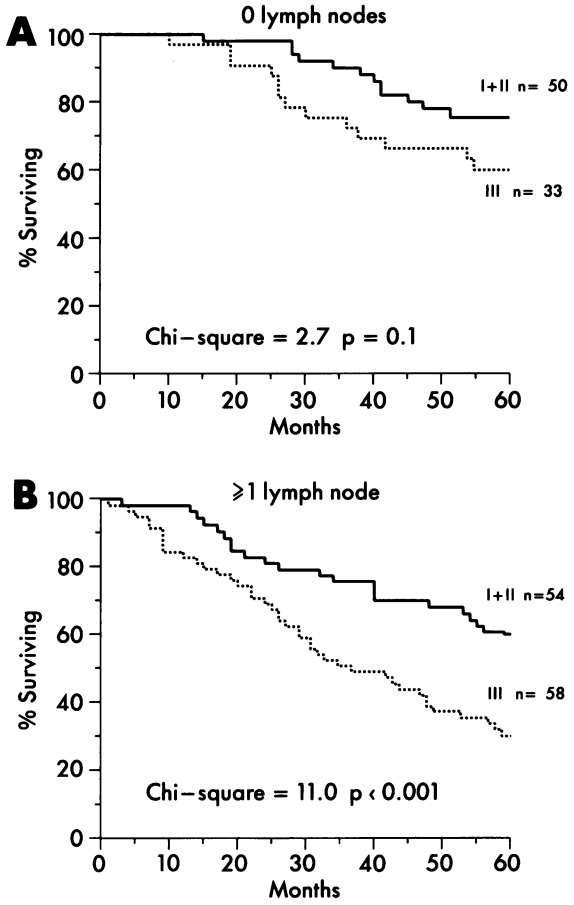

Figure 2. *Effect of histologic grade on survival curves of patients with infiltrating ductal NOS breast carcinomas.* **A**: *Node-negative cases.* **B**: *Cases with involvement of one or more axillary lymph nodes. I + II: histologic grades, I, II, II/III. III: histologic grade III.*

Table 3. *Coefficients of Correlation Between Survival, Histologic Grade, Tumor Size, Lymph Nodes Involved, and Vimentin in Ductal NOS Breast Carcinomas*

	Months	Grade	Size	Nodes	Vimentin
A All Patients (n = 195)					
M	1.00				
G	0.27	1.00			
S	0.27	0.05	1.00		
N	0.49	0.21	0.26	1.00	
V	0.19	0.20	0.00	0.01	1.00
All	46.66	1.43	44.49	2.84	0.17
V−	48.22	1.37	44.50	2.82	0.00
V+	39.24	1.68	44.41	2.91	1.00
B At Least One Node Affected (n = 112)					
M	1.00				
G	0.30	1.00			
S	0.29	−0.04	1.00		
N	0.51	0.22	0.27	1.00	
V	0.10	0.11	−0.08	0.28	1.00
All	42.63	1.49	46.96	4.94	0.11
V−	43.31	1.47	47.45	4.54	0.00
V+	36.92	1.67	42.92	8.25	1.00
C No Nodes Affected (n = 83)					
M	1.00				
G	0.17	1.00			
S	0.15	0.12	1.00		
N	0.00	0.00	0.00	0.00	
V	0.51	0.35	0.15	0.00	1.00
All	52.10	1.34	41.14	0.00	0.27
V−	56.28	1.21	39.67	0.00	0.00
V+	40.50	1.68	45.23	0.00	1.00

Table 3A–C give the coefficients of correlation between the prognostic variables grade (1, 2, 3), size of tumor (in mm), number of nodes affected, and vimentin (0 = negative, 1 = positive) and survival in months after diagnosis (censored to 60 months, coefficients taken positive). The correlations are given in A for all patients, in B for those with at least one affected lymph node, and in C for those without affected lymph nodes, n = patients at risk. At 95% significance by *t*-test, correlations of 0.14, 0.185, and 0.215 are required as evidence against the hypothesis of noncorrelation for n = 195, 112, and 83, respectively. We list the average values of each parameter overall in the group and segregated for V− and V+ below the correlation coefficients in each group.

Discussion

Prognosis in Breast Carcinoma

Metastatic tumor in axillary lymph nodes has long been regarded as the most powerful indicator of poor prognosis in breast carcinomas.[15] High nuclear or histologic grade of the tumor[16] and increased proliferative activity as measured by the size of S-phase fraction by means of thymidine labeling index[7,9] also have been correlated with poor prognosis. Histologic type of the tumor also plays a role because prognosis in ductal carcinomas is known to be worse than, for instance, in medullary cancer.[17,18] Recently biologic grading of breast cancer using antibodies specific to proliferating cells and to other markers provided additional parameters that may be used to assess prognosis in breast carcinoma. These include Ki-67 antibody[6,19,20] and antibody to bromodeoxyuridine,[21] which detect growth fraction and S-phase fraction, respectively, estrogen receptor (ER)[10,22,23] and epidermal growth factor receptor (EGFR) status,[8] as well as expression of the c-erbB-2 (or neu/HER2) oncoprotein.[24] Quantitation of immunohistochemical staining by image analysis is an additional advantage of these markers because it provides an objective method of assessment.[25]

Vimentin and Prognosis

Vimentin expression in breast carcinoma was linked originally to three biological poor prognostic indicators, ie, low estrogen receptor level,[1,2] positive EGFR status,[2] and high proliferative activity of the tumor[1,4] and one histologic indicator of poor prognosis, ie, high histologic grade of ductal NOS carcinomas.[1,3–5] Recently, in two studies of renal cell carcinomas, a correlation between vimentin expression and high nuclear grade was found,[26,27] with a particularly unfavorable course for vimentin-positive nuclear grade 3 tumors. Therefore we[1,5] and Raymond and Leong[4] suggested that vimentin might be an indicator of poor prog-

nosis in a subset of infiltrative ductal NOS breast carcinomas. The results of the current study suggest that vimentin expression is indeed associated with poor prognosis in node-negative ductal NOS breast cancer. The eight node-negative patients with ductal NOS carcinomas in this series who died of cancer within 27 months had tumors that expressed vimentin. Only 36% of patients with vimentin-positive tumors survived 5 years, as compared to 82% of those who had vimentin-negative tumors. Thus vimentin seems to join the list of biological markers that are associated with poor prognosis in node-negative ductal NOS breast carcinomas. However because we have found vimentin expression in tumor cells of 78% of medullary and in two of four mucinous breast carcinomas,[5] we again make the point that vimentin *per se* cannot be regarded as a predictor of behavior for *all* types of breast carcinomas, although it is more likely a feature of tumors undergoing rapid growth (for further discussion of vimentin expression and differences in biological behavior between ductal NOS and medullary carcinoma, Domagala et al[5]).

Our data suggest that vimentin may be a convenient predictor of biological behavior for node-negative ductal NOS breast carcinomas. While some markers (eg, Ki-67) require fresh frozen tissue, vimentin expression can be assessed on formalin- (or alcohol-) fixed paraffin-embedded breast tumors if an appropriate vimentin antibody is selected. Vimentin, like ER or Ki-67 growth fraction, also may be assayed on fine-needle aspirates of breast tumors[1,28–31]; hence the prognostic information it carries may be available before surgical treatment.

In the node-negative breast carcinomas, ideally adjuvant therapy should be reserved for patients at high risk of relapse.[32,33] The current difficulty, however, is to know which factors are most useful in identifying this group of patients so that recommendations can be made for treatment. Our data suggest that vimentin expression in node-negative ductal NOS breast cancer is significantly associated with poor survival at 5 years. Therefore vimentin seems to be a good prognostic factor because it is easily measurable and allows wide separation of prognostic groups. Further research is needed to determine whether vimentin and/or other strong poor prognostic indicators, such as Ki-67 growth fraction and EGFR status, can serve as discriminating factors in treatment decisions in node-negative ductal NOS breast cancer patients.

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Acknowledgment

The authors thank Susanne Isenberg for help in staining the specimens.