ANTICANCER RESEARCH *25*: 2879-2884 (2005)

E-cadherin and Fibronectin Expressions Have No Prognostic Role in Stage II Ductal Breast Cancer

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Abstract. *Background: The aim of the study was to describe the expressions of E-cadherin (EC) and fibronectin (FN) and their prognostic value in radically-treated ductal breast cancer. Patients and Methods: Ninety-eight patients, aged 26-86 (median, 57) with stage II G2 or G3 tumors, were subjected to a retrospective analysis. Results: No significant associations were observed between the levels of the proteins studied and patient or cancer features, with the exception of relationships between FN expression and the histological grade of the tumor or hormonal status of the subjects. None of the markers showed a correlation with survival in multivariate analysis. Consequently, the experiment revealed no prognostic value of EC or FN expression in a homogenous patient group with stage II G2 or G3 ductal breast carcinoma. Conclusion: Further studies, on other uniform populations, with tumor features different from those described here, are necessary in order to reveal the prognostic significance of the molecules discussed.*

While the prognosis and need for further treatment is obvious in the extreme (I, III and IV) stages of breast cancer, attitudes regarding stage II cases are still conflicting. It is estimated that only a small percentage of stage II patients benefit from aggressive chemotherapy. Consequently, it is of major importance to define the immunohistochemical features of this group to enable the stratification of patients and treatment individualization.

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Key Words: Breast cancer, ductal breast cancer, E-cadherin, fibronectin, prognosis.

Altered expression of adhesion molecules has been implicated in invasive and metastatic malignant growth (1, 2). Abnormalities of the E-cadherin/catenin complex play an important role in disorders of cell-cell adhesion observed in epithelial malignant neoplasms, including breast cancer. Cadherins are calcium-dependent transmembrane molecules. Their intracellular domains bind to catenins – proteins exhibiting variable activity in the course of the normal cell cycle and in neoplastic transformation (3-5). The negative prognostic value of E-cadherin (EC) expression loss in breast cancer has been revealed by many authors (6-9).

Integrins are molecules which play a significant role in cell-extracellular matrix (ECM) interactions. They interact with the RGD tripeptide of fibronectin (FN), one of the main components of ECM. Labile expression of FN has been proven to play an important role both in the normal developmental process (morphogenetic movements) and in the course of carcinogenesis (10-12).

Considering the aforementioned role of the molecules discussed, the aim of this study was to describe the expressions of EC and FN and their prognostic value in radically-treated ductal breast cancer.

Patients and Methods

Patients. Archival tumor samples from 98 patients of the Lower Silesian Oncology Center, Wroclaw, Poland, treated radically for stage II ductal breast cancer from 1993-1996 were studied. The median age of the patients was 57 (range, 26-86 years) and detailed patient characteristics are given in Table I. All the patients had undergone surgery (radical Patey mastectomy) with or without adjuvant treatment. Overall survivals (OS, in weeks) and disease-free survivals (DFS, in weeks) were established for all the patients. The follow-up period amounted to 5 years (261 weeks). The type of relapse (local only or local + dissemination) was defined in patients with recurrence (n=23, 23.5%). Microscopic studies were

Table I. *Patient characteristics related to the results of E-cadherin and fibronectin immunostaining.*

Patient characteristics	Total		E-cadherin expression						*p*	Fibronectin expression						*p*
			Low		Intermediate		High			Low		Intermediate		High		
	n	%	n	%	n	%	n	%		n	%	n	%	n	%	
Age																
≤50 years	31	31.6	12	38.7	13	41.9	6	19.4	0.641	16	51.6	9	29.0	6	19.4	0.207
>50 years	67	68.4	32	47.8	17	25.4	18	26.9		45	67.2	17	25.4	5	7.4	
Hormonal status																
premenopausal	30	30.6	14	46.7	9	30.0	7	23.3	0.826	14	46.7	10	33.3	6	20.0	0.074
postmenopausal	68	69.4	30	44.1	21	30.9	17	25.0		47	69.1	16	23.5	5	7.4	
TMN stage																
IIa	40	40.8	17	42.5	14	35.0	9	22.5	0.950	28	70.0	8	20.0	4	10.0	0.243
IIb	58	59.2	27	46.5	16	27.6	15	25.9		33	56.9	18	31.0	7	12.1	
Histological grade																
G2	67	68.4	30	44.8	18	26.9	19	28.3	0.426	38	56.7	20	29.9	9	13.4	0.032
G3	31	31.6	14	45.2	12	38.7	5	16.1		23	74.2	6	19.4	2	6.4	
Lymph node metastasis																
absent	58	59.2	26	44.8	18	31.0	14	24.2	0.971	37	63.8	18	31.0	3	5.2	0.223
present	40	40.8	18	45.0	12	30.0	10	25.0		24	60.0	8	20.0	8	20.0	
Relapse																
absent	75	76.5	32	42.7	25	33.3	18	24.0	0.770	47	62.7	21	28.0	7	9.3	0.622
local	3	3.1	2	66.7	1	33.3	0	0.0		2	66.7	1	33.3	0	0.0	
disseminated	20	20.4	10	50.0	4	20.0	6	30.0		12	60.0	4	20.0	4	20.0	
TOTAL	98	100.0	44	44.9	30	30.6	24	24.5		61	62.3	26	26.5	11	11.2	

performed on formalin-fixed, paraffin-embedded cancer tissues, obtained during surgery and stained routinely with hematoxylin and eosin. The histopathological type according to the World Health Organization (ductal breast cancer in all the cases), grade (only G2 and G3 were enlisted) and stage according to the TNM classification were determined during microscopic examination.

Immunohistochemistry. Sections from formalin-fixed, paraffin-embedded blocks were immunostained for EC, FN and estrogen receptor (ER) using the Biotin-Streptavidin-Peroxidase method. Deparaffinized and rehydrated sections were incubated with citrate buffer at 98˚C to unmask the epitopes and treated with 1% hydrogen peroxide (H_2O_2) for 10 min to block endogenous peroxidase. The sections were then incubated with monoclonal antibodies against EC (Clone NCH-38, DakoCytomation, Glostrup, Denmark), FN (A 0245, DakoCytomation) or ER (Clone 105, DakoCytomation) overnight at room temperature. The sections were then incubated with biotin-labelled secondary antibody and streptavidin-biotin-peroxidase for 20 min each. The tissue was stained for 5 min with 0.05% 3,3'-diaminobenzidine tetrahydrochloride (DAB) and then counterstained with hematoxylin, dehydrated and mounted. The expressions of the molecules studied were graded using a semiquantitative method, scoring either the area (no staining=0, <10%=1, 10-50%=2, 51-75%=3, >75%=4) or intensity (no staining=0, low=1, intermediate=2, strong=3) of the color reaction, with the final result being a product of both the variables.

Statistical analysis. The association between the expressed proteins and between the protein expressions and clinicopathological parameters was tested by the Mann-Whitney *U*-test. The Cox proportional hazards regression model was used for multivariate analysis of survival. The following parameters were considered: age and hormonal status of patients, tumor stage according to the TNM classification (IIa *vs.* IIb), axillary lymph node metastasis, histological grade (G2 *vs.* G3), type of relapse (local or disseminated), 5-year overall survivals (OS), 5-year disease-free survivals (DFS), rates of FN, EC and ER expression. The Statistica 5, Version 97 (StatSoft®, Poland) statistical package was used for the statistical analysis and statistical significance was defined as $p<0.05$.

Results

The staining results for EC and FN in ductal breast cancer are summarized in Table II. The expressions of EC and FN were low (score ≤4, Figure 1A and 2A) in 44/98 and 61/98

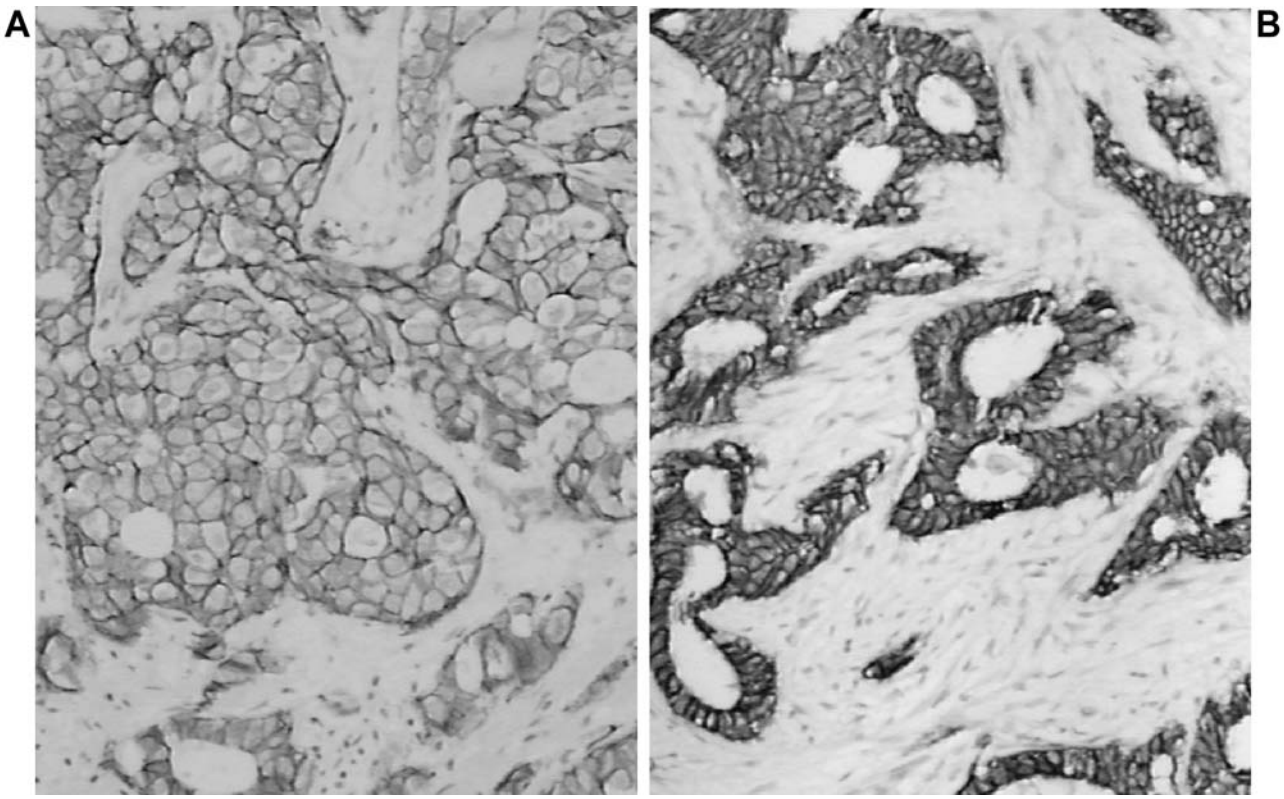

Figure 1. *Low (A) and high (B) immunohistochemical staining of E-cadherin in ductal breast cancer.*

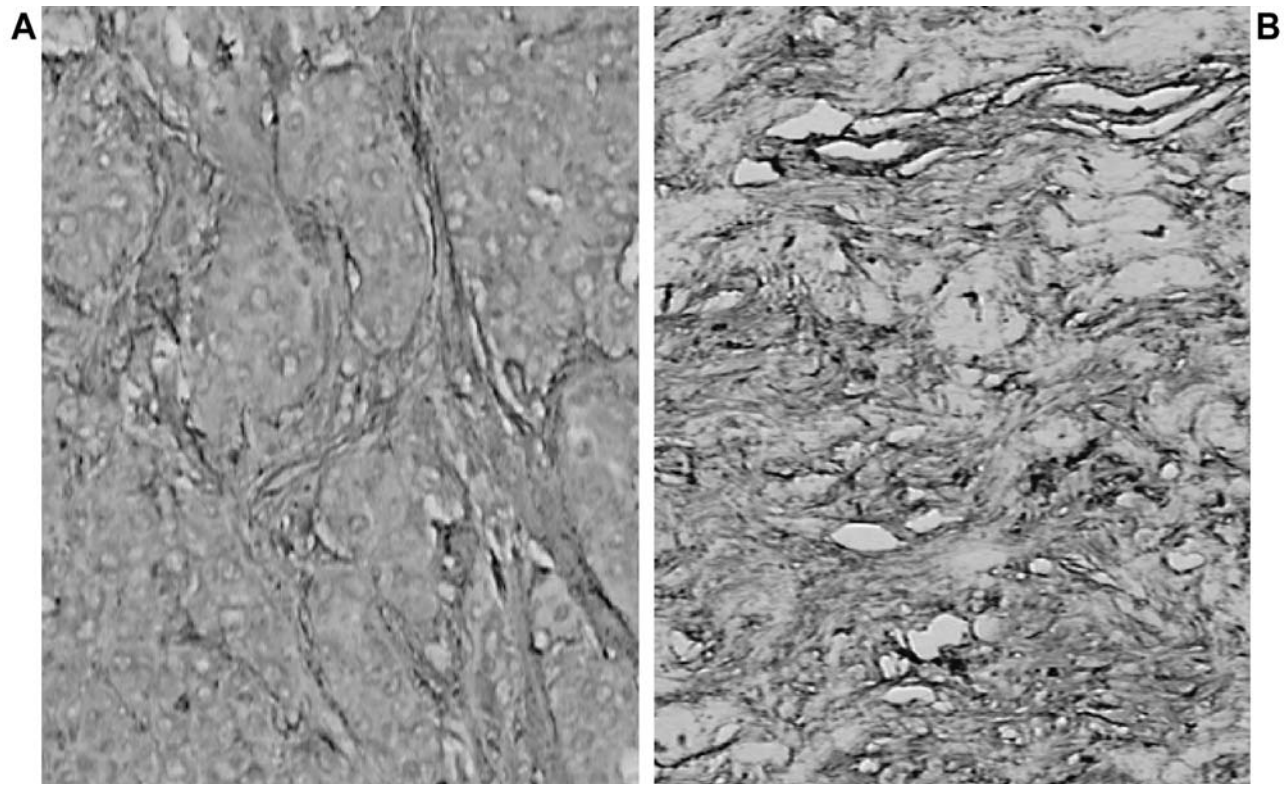

Figure 2. *Low (A) and high (B) immunohistochemical staining of fibronectin in ductal breast cancer.*

Table II. *Levels of E-cadherin and fibronectin expression in primary tumors.*

Level of expression	E-cadherin		Fibronectin	
	n	%	n	%
0	5	5.10	1	1.02
1	7	7.14	9	9.18
2	12	12.24	14	14.28
3	4	4.08	-	-
4	16	16.32	36	36.73
6	27	27.55	27	27.55
8	3	3.06	-	-
9	14	14.28	7	7.14
12	10	10.20	4	4.08

tumors, respectively, and high (score ≥9, Figure 1B and 2B) in 24/98 and 11/98 tumors, respectively.

The immunostaining frequencies of the molecules studied in relation to patient features and clinicopathological tumor parameters are provided in Table I. The only significant association was between FN expression and histological grade ($p=0.032$). Moreover, a high but insignificant correlation was demonstrated between the FN level and the hormonal status of the patients ($p=0.074$). Statistical relationships were not observed between the rates of EC or FN expression and patient age, TNM tumor stage, local lymph node metastasis or type of relapse.

Regarding tumor expression, EC and FN showed no positive correlation to each other ($p=0.21$). Moreover, none of the markers for adhesion showed correlation with survival (OS or DFS) in multivariate analysis.

Discussion

Many authors have implicated loss or decrease of EC expression as an independent negative prognostic marker in breast cancer patients (6-9). There is increasing experimental evidence for a relationship between the EC level and different features of breast cancer, including histological grade (7, 16) and axillary lymph node involvement (13-16). However, some reports indicated a lack of relationship between a low expression of EC and poorer prognosis (17), histological grade or axillary lymph node metastasis (18). Our study did not reveal any significant relationship between EC expression and the properties of breast cancer. In contrast to other studies, our experimental group was quite homogenous (stage II ductal breast carcinoma). The lack of prognostic value of EC expression might also be related to the relatively short follow-up period (5 years) in the case of breast cancer. It is very likely that prolonged follow-up of such a homogenous group of patients would reveal the prognostic value of decreased EC expression.

Studies on alterations of FN expression in breast cancer are scant and conflicting. Extracellular FN expression has was proved to be positively correlated with lymph node involvement and seemed to have negative prognostic value in breast cancer patients (19), however, this observation was not confirmed by the present study. Takei *et al.* (20) revealed that FN expression does not correlate with lymph node metastasis or tumor size. Moreover, these authors found FN overexpression to be an independent predictor of relapse-free survival in invasive breast carcinoma patients, a finding inconsonant with the results of our study. Matsumoto *et al.* (21) observed increased FN expression in invasive ductal carcinomas, although absent in fibroadenomas and other benign conditions of human breast tissues. The authors consequently suggested a potential role for that molecule in breast cancer progression. A higher expression of FN was, however, related to lower histological grade (G2 *vs.* G3) in our study ($p=0.032$). Moreover, increased expression of the protein studied was noted in premenopausal patients, suggesting that FN might be involved in breast cancer pathogenesis at the stages preceding its spread.

In conclusion, our experiment revealed no prognostic value for EC or FN expressions in a homogenous group of patients with stage II G2 or G3 ductal breast carcinoma. Accordingly, further studies, on other selected, homogenous populations, with tumor parameters other than those described here, are necessary in order to reveal the prognostic significance of these molecules.

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Received February 16, 2005
Accepted May 5, 2005