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.
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 (H2O2) 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.
survival (OS or DFS) in multivariate analysis. Correlation of the markers for adhesion showed correlation with 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 homogeneous (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 been 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 inconsistent 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.

**References**


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

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