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Breast cancer expression of E-cadherin does not differ between patients with positive and negative oncological history

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ABSTRACT

Purpose: The question of whether or not non-sporadic breast malignancies have different immunohistochemical features than sporadic malignancies has not been investigated previously. Consequently, the purpose of this study was to compare the expression of E-cadherin (EC) in breast cancer patients with positive and negative oncologic histories.

Material and Methods: The study included 98 breast cancer patients divided into two groups: 1) without the personal or familial history of previous malignancies, and 2) with the personal history of previous malignancies and/or with the data on cancer episodes in first- and/or second-degree relatives.

Results: There were no significant differences in the expression of EC between breast malignancies of the two groups. Moreover, statistical relationships were not observed between the positive or negative oncologic history, the age, and the menopausal status of patients, or histological tumor grade.

Conclusions: Although the results of our series revealed no significant differences in the expression of EC between assumed sporadic and assumed non-sporadic malignancies, there is a need for further comparative studies on the immunohistochemistry of both the breast carcinoma types in order to find the other biological markers that could suggest or exclude cancer susceptibility in a given patient. Nevertheless, the results of our study suggest that EC immunohistochemistry cannot be used as a surrogate marker for screening for hereditary breast cancer.

Key words: E-cadherin, ductal breast cancer, oncological history

INTRODUCTION

It is currently estimated that 5 to 10% of all breast cancers are hereditary and attributable to mutations in several susceptibility genes, some of which have been identified, including: *BRCA1*, *BRCA2*, *PTEN* and *p53* [1,2]. So it is of major importance to define the immunohistochemical features of this group of neoplasms to carry out genetic testing more effectively and also to gain insight into the biological characteristics of the tumors.

The disorders of cell-cell adhesion, related inter alia to the abnormalities of the E-cadherin/catenin complex, are known to play an important role in the pathogenesis of several malignancies, including breast cancer [3,4]. 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 the neoplastic transformation [5-9]. The somatic mutations of E-cadherin gene, *CDH1*, have been implicated in the carcinogenesis of familial gastric, colorectal and breast cancer [10-14]. Recent findings, however, do not support *CDH1* as a breast cancer susceptibility gene [15-17].

Table 1. **Combined immunoreactive E-cadherin scores and other characteristics of tumor and patient, stratified by oncologic history.**

	Total		Group 1		Group 2		P
	n	(%)	n	(%)	n	(%)	
Age							
≤50 years	31	(31.6)	21	(33.3)	10	(28.6)	0.629
>50 years	67	(68.4)	42	(66.7)	25	(71.4)	
Hormonal status							
premenopausal	30	(30.6)	20	(31.7)	10	(28.6)	0.243
postmenopausal	68	(69.4)	43	(68.3)	25	(71.4)	
Histological grade							
G2	67	(68.4)	42	(66.7)	25	(71.4)	0.629
G3	31	(31.6)	21	(33.3)	10	(28.6)	
E-cadherin expression							
≤4	44	(44.9)	26	(41.3)	18	(51.4)	0.312
5-8	30	(30.6)	20	(31.7)	10	(28.6)	
≥9	24	(24.5)	17	(27.0)	7	(20.0)	
TOTAL	98	(100.0)	63	(64.3)	35	(35.7)	

According to our knowledge, the question of whether or not non-sporadic, *BRCA1/2*-related breast malignancies have different immunohistochemical features than sporadic malignancies has not been previously investigated. The only exceptions are the immunohistochemical features of the "basal-like phenotype" of ductal carcinoma, which have recently been extensively described. This phenotype was found to be associated – though not specific for – with *BRCA1*-associated tumors [18].

E-cadherin mutations have already been proved to result in invasive carcinomas (diffuse gastric carcinoma and lobular mammary carcinomas) and familial gastric carcinomas are in fact associated with germ line *CDH1* mutations [12]. Therefore, it could be hypothesized that such mutations may play a role in breast carcinogenensis. Consequently, the aim of this study was to use immunohistochemistry as a surrogate marker for mutations in the E-cadherin gene and compare its expression among subgroups of patients according to cancer history.

MATERIALS AND METHODS

Archival tumor samples from 98 patients of the Lower Silesian Oncology Center, Wroclaw, Poland, treated radically for stage II ductal breast cancer between 1993 and 1996 were studied.

Considering the detailed data from medical history, the patients were divided into two groups: 1) 63 breast cancer patients without the personal or familial history of previous malignancies, and 2) 35 breast cancer patients: a) with the personal history of previous malignancies (n=5), b) with the data on cancer episodes in at least one first- and/or second-degree relative at the age <50 years (n=27), or c) with both familial and personal history of cancer (n=3).

Sections from formalin-fixed, paraffin-embedded blocks were immunostained for EC using the avidin-biotin-peroxidase method [19]. Deparaffinized in xylene and rehydrated in ethanol, sections were incubated with a citrate buffer at 98ºC to unmask the epitopes (20 min) and treated with 1% hydrogen peroxide (H_2O_2) for 10 min to block endogenous peroxidase. The sections were then incubated overnight at room temperature with a monoclonal antibody against EC (Clone NCH-38, DakoCytomation, Glostrup, Denmark) at a 1:150 dilution. The sections were then incubated with a biotin-labeled secondary antibody and avidin-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 haematoxylin, dehydrated and mounted.

The expression of EC was graded using a semiquantitative method, scoring the percentage of reactive cells (no staining = 0, <10 % = 1, 10-50 % = 2, 51-75 % = 3, >75 % = 4) and the intensity (no staining = 0, weak = 1, intermediate = 2, strong = 3) of the color reaction, with the final result being a product of both variables. Consequently, nine possible products (0, 1, 2, 3, 4, 6, 8, 9 and 12) were obtained, which were considered as low (0, 1, 2, 3, 4), medium (6 and 8) or high (9 and 12) expressions of EC in further statistical analysis.

The association between the EC expression, patient features, or clinicopathological tumor parameters and oncologic history was tested by the Mann-Whitney (U) test. The Statistica 5, Version 97 (StatSoft®, Poland) statistical package was used for the statistical analysis and the statistical significance was defined as $P \leq 0.05$.

Figure 1a. **Low immunohistochemical staining of E-cadherin in ductal breast cancer.**

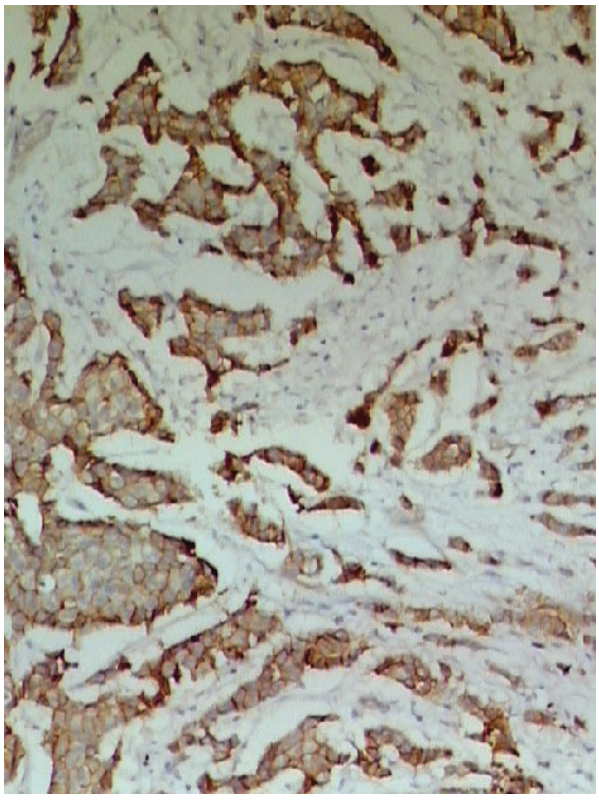
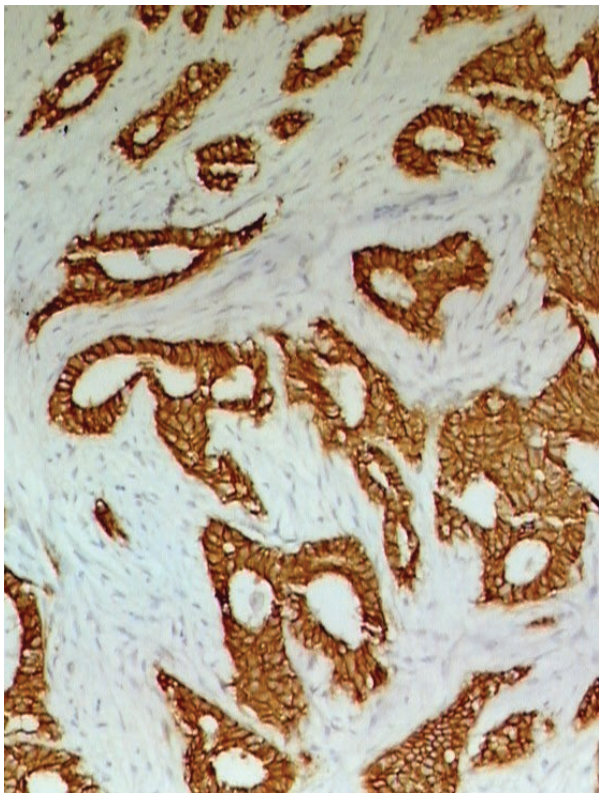


Figure 1b. **High immunohistochemical staining of E-cadherin in ductal breast cancer.**



RESULTS

Samples from 98 stage II ductal breast cancer patients were studied. The median age of the patients was 57 (range, 26-86 years). Thirty patients were premenopausal (30.6%) and 68 (69.4%) were postmenopausal. Axillary lymph nodes were positive in 40 (40.8%) women.

The staining results for EC in ductal breast cancer are summarized in *Tab. 1*. The expression of EC was low (score ≤4, *Fig. 1a*) in 26/63 (41.3%) and 18/35 (51.4%) patients from group 1 and 2, respectively, and high (score ≥9, *Fig. 1b*) in 17/63 (27.0%) and 7/35 (20.0%) patients from group 1 and 2, respectively. Regardless of staining intensity, all specimens showed the membranous pattern of staining.

Combined immunoreactive E-cadherin scores and the other characteristics of tumor and patient, stratified by oncologic history, are shown in *Tab. 1*. Statistical relationships were not observed between the positive or negative oncologic history and the score of the EC expression, patient age and menopausal status, or histological tumor grade.

Finally, there was no significant relationship if the EC scores were stratified by grade, both for the whole cohort (p=0.312) and the individual groups (p=0.552 and p=0.495 for groups 1 and 2, respectively, *Tab. 2*). There was a predominance of low EC expression, regardless of tumor grade.

DISCUSSION

In our series, the breast cancer expression of EC was not significantly different between the patients with negative and positive oncological history. The latter group included patients with assumed individual and/or familial predisposition to cancer.

Since germ-line mutations in EC have been linked to familial and early-onset gastric carcinoma, it is plausible that such mutations may contribute to heritable forms of breast cancer. However, the only study which has shown this relationship considered a single woman, who had a metachronous development of lobular breast and diffuse type gastric carcinomas. Immunohistochemistry for the EC expression revealed an abnormal staining pattern in both of these tumors, suggesting complete inactivation of the cell adhesion molecule [12]. Recent studies, carried out on larger populations of patients, have failed to demonstrate E-cadherin germ-line mutations in patients with breast cancer. Rahman et al. [15] examined a series of 65 patients with *in situ* lobular carcinoma for germ-line mutations in *CDH1* gene. Four polymorphisms were detected but no pathogenic mutations were identified. Consequently, *CDH1* was found unlikely to act as a susceptibility gene for lobular breast cancer. Salahshor et al. [16] analyzed patients with sporadic (n=614)

Table 2. **Combined immunoreactive E-cadherin scores stratified by tumor grade.**

	Grade	E-cadherin expression								P
		≤4		5-8		≥9		Total		
		n	%	n	%	n	%	n	%	
Total (n=98)	G2	30	(44.8)	18	(26.9)	19	(28.3)	67	(100.0)	0.312
	G3	14	(45.2)	12	(38.7)	5	(16.1)	31	(100.0)	
Group 1 (n=63)	G2	17	(40.5)	12	(28.5)	13	(31.0)	42	(100.0)	0.552
	G3	9	(42.9)	8	(38.1)	4	(19.0)	21	(100.0)	
Group 2 (n=35)	G2	13	(52.0)	6	(24.0)	6	(24.0)	25	(100.0)	0.495
	G3	5	(50.0)	4	(40.0)	1	(10.0)	10	(100.0)	

or familial (n=484) breast cancer and 497 control individuals for missense mutation in exon 12 of the EC gene, previously found in one family with diffuse gastric cancer and colon and breast carcinomas. The frequencies of alteration studied were similar in particular groups (0.83%, 0.68% and 0.80% for the sporadic, familial and control group, respectively). However, a correlation between the alteration and invasive ductal comedo-type tumor was seen, suggesting that the germline mutation in *CDH1* influences the behavior of the tumor, rather than predisposes to breast cancer. Also, the results of Lei et al. [17] do not support *CDH1* as a prominent low-penetrance cancer susceptibility gene, but indicate that its mutations contribute to the progression of both lobular and ductal breast tumors. It is of interest, however, to compare E-cadherin mutations in invasive ductal carcinomas versus invasive lobular carcinomas. Frequent LOH at *16q* in both cancers suggest a common mutation in a yet to be identified tumor suppressor gene at this locus. The hypothesis that *CDH1* is the tumor suppressor gene at this locus has been explored in other studies [20].

Although the aforementioned studies dealt with role of *CDH1* in breast cancer development, their findings are somehow consistent with our observations on the expression of E-cadherin gene product in sporadic and non-sporadic malignancies. Consequently, the pathogenesis of breast cancer seems to be similar in patients with positive and negative oncologic history, at least in relation to the EC expression.

CONCLUSIONS

Although the results of our series revealed no significant differences in the expression of EC between assumed sporadic and assumed non-sporadic malignancies, there is a need for further comparative studies on the immunohistochemistry of both of the breast carcinoma types, in order to find the other biological markers that could suggest or exclude cancer susceptibility in a given patient. Nevertheless, the results of our study suggest that the EC immunohistochemistry cannot be used as a surrogate marker for screening for hereditary breast cancer.

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