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E-Cadherin as a diagnostic biomarker in breast cancer

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Abstract

Background: E-cadherin is expressed in most normal epithelial tissues. Selective loss of E-cadherin can cause dedifferentiation and invasiveness in human carcinomas, leading E-cadherin to be classified as a tumor suppressor. Loss of E-cadherin has been demonstrated in invasive lobular carcinoma of the breast, but the relationship between E-cadherin expression and breast cancer histopathology and prognosis is less clear. **Aim:** Our objective was to assess loss of E-cadherin as a diagnostic breast cancer biomarker and as an aid to the sub-classification of invasive breast cancer. We also correlated the loss of expression of E-cadherin with various clinical and pathologic prognostic factors. **Material and Methods:** Breast cancer specimens after modified radical mastectomy were obtained from women who underwent surgery at Grant Medical College and Sir J.J Group of Hospitals, Mumbai, India between May 2007 and October 2010. We stained 276 breast cancers specimens with monoclonal antibodies to E-cadherin. The breast cancers were classified by histopathological type. **Results:** A statistical correlation of E-cadherin loss with a positive diagnosis of invasive lobular carcinoma was found, but there was no correlation with any prognostic tumor variables. A negative E-cadherin stain was a sensitive and specific biomarker to confirm the diagnosis of invasive lobular carcinoma (specificity 97.7%; negative predictive value 96.8%; sensitivity 88.1%; and positive predictive value 91.2%). Positive E-cadherin expression was also associated with tubulolobular carcinomas. **Conclusions:** E-cadherin immunohistochemistry is helpful in classifying breast cancer cases with indeterminate histopathologic features. E-cadherin loss is uncommon in non-lobular carcinomas but shows no correlation to currently established prognostic variables.

Keywords: Breast cancer, E-Cadherin, prognostic cancer tissue biomarkers, invasive lobular carcinomas, invasive ductal carcinoma, tubulolobular carcinoma, invasive carcinoma.

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Introduction

E-cadherin is a calcium-regulated adhesion molecule expressed in most normal epithelial tissues [1]. The E-cadherin gene is located on chromosome *16q22.1* [2]. E-cadherin is associated with gland formation, stratification, and epithelial polarization [3]. E-cadherin knockout mice are non-viable and have abnormal epithelial morphogenesis [4]. Selective loss of E-cadherin can cause dedifferentiation and invasiveness in human carcinomas [4, 5]. In various cell lines, a reciprocal relationship has been shown between levels of E-cadherin expression and invasiveness [5]. Reduced expression of E-cadherin has been observed in aggressive tumors of the esophagus, ovary, and stomach [6-8]. Mechanisms by which E-cadherin protein expression is lost include E-cadherin gene mutation and loss of the wild-type allele by loss of heterozygosity [9-11]. These data indicate that E-cadherin is a classic tumor suppressor gene [9, 12].

Ductal and lobular carcinomas of the breast represent the main infiltrating carcinomas, the latter being less frequent. Traditionally, histopathologic features have been used to classify mammary carcinomas. Although the established histopathologic criteria distinguish invasive lobular from invasive ductal carcinoma, diagnostic difficulty occurs because of overlapping histopathologic features,

particularly with invasive lobular carcinoma (ILC) variants and pleomorphic ILC [13-17]. Proper histopathologic categorization of breast carcinomas has prognostic implications [18].

The majority of ILCs have shown a complete loss of E-cadherin expression [17-22]. The loss of E-cadherin is from the outset, i.e., in the pre-invasive stage of lobular carcinoma in situ (LCIS). E-cadherin loss explains the histopathologic appearance of LCIS including a diffuse growth pattern of this non-gland-forming tumor with discohesive tumor cells [23].

However, the practical application of E-cadherin expression in breast cancer as a prognostic and diagnostic cancer biomarker remains controversial. Reduced E-cadherin expression was an adverse prognostic biomarker in some studies [24-27]. Although most studies show reduced expression of E-cadherin to be associated with high histopathologic grade [20, 21, 25, 27, 28], correlation with nodal metastasis [29] and loss of estrogen receptor (ER) and progesterone receptor (PgR) [27, 28] have been shown in only some studies. With the exception of histopathologic grade, the relationship between E-cadherin expression in regard to different prognostic markers and survival differs between studies [24, 30].

We evaluated E-cadherin expression as an aid to sub-classification of invasive breast cancer. In addition, we correlated the loss of expression of E-cadherin with various clinical and pathologic prognostic factors. While correlating the prognostic criteria with E-cadherin loss, we considered the inherent loss of E-cadherin in all lobular breast carcinomas, irrespective of their histopathologic grade, and the expression of other prognostic tumor variables that previous studies have not considered.

Materials and Methods

Human breast tumor tissue collection and histopathology

We collected 276 breast cancer specimens from women undergoing modified radical mastectomy for operable primary breast cancer between May 2007 and July 2010. All breast cancer tissues were collected from surgeries performed at Grant Medical College and Sir J.J Group of Hospitals, Mumbai, India.

Histopathology was based on hematoxylin and eosin (H&E) stained slides. The pathology specimens were reviewed independently by histopathologists to grade and sub-classify the tumors based on established criteria [13, 14] without knowledge of immunohistochemical results. Discrepancies in diagnoses were resolved by consensus with simultaneous viewing. All invasive carcinomas were graded using the Nottingham grading system of Elston and Ellis [31].

After final histopathologic review, 276 breast cancer cases were further studied, including 204 cases of invasive ductal carcinoma (IDC) and ductal special types (tubular, mucinous); 59 cases of ILC and variants (49 conventional and 10 pleomorphic ILC); 4 cases of tubulolobular carcinoma (TLC); and 9 cases of invasive carcinoma (IC), with uncertain classification between lobular and ductal type. Data on patient demographics, tumor size, axillary lymph node status, stage of disease, ER and PgR status, and HER-2/neu overexpression were abstracted from the histopathology reports.

Immunohistochemistry

Tissue samples were fixed in 10% neutral buffered formalin for 12 - 24 hours. Tissue samples were processed in an auto processer and embedded in paraffin wax on an embedding station. The tissue blocks were sectioned by microtome into 4 μM sections that were dried overnight at 37°C. Prior to antibody staining, the slides were pre-treated with microwave irradiation to unmask binding epitopes. After blocking endogenous peroxide activity with a 3% solution of hydrogen peroxide in methanol for 30 minutes, slides were immersed in 200 mL of 10 mM citric acid (pH 6.0) for 5 minutes at 100 Watt power in a microwave oven, followed by 4 cycles of 5 minute each on 50 Watt power. After topping up the buffer with distilled water, these steps were repeated. The slides were then left to stand for 10 minutes in buffer at room temperature before being washed thoroughly in tap water.

After three washes in tris-buffered saline (TBS), the slides were incubated with a 1:50 dilution of mouse anti-E-cadherin monoclonal primary antibody (Clone: NCH-38; M3612; DakoCytomation, Denmark) in TBS for 1 hour at room temperature. After three more washes in TBS, the secondary antibody, biotinylated goat antibody (LINK) to mouse/rabbit immunoglobulin (K0355; DakoCytomation, Denmark) at a dilution of 1:100 in TBS was applied for 1 hour at room temperature. After an additional three washes, a streptavidin-biotin-horseradish peroxidase (HRP) complex (Enzyme Label) (K0355; DakoCytomation, Denmark) was formed. After an additional three washes, the staining was visualized by adding diaminobenzidine (DAB) (K3467; DakoCytomation, Denmark) for 5 minutes at room temperature. The slides were washed well in tap water and counterstained with Harris's hematoxylin for 10 seconds to 1 minute and then dehydrated, cleared, and mounted in Distrene Plasticiser Xylene (DPX). Positive and negative controls were performed with each batch of slides. Surgical specimens from the same patient were stained on the same run.

The entire stained slide was evaluated for immunostaining by light microscopy. Image collection and microphotographs were taken with an AxioImager M1 Microscope with AxioVision software (Carl Zeiss Microscopy, Germany). The slides were first observed under a 10X objective to confirm that the cells were still attached to the slide. Final evaluation was performed under 400X objective magnification. All images were taken under 400X objective magnification without oil immersion lens. All images were processed with AxioVision software.

Table 1 Patient age, tumor histopathologic grade, and E-cadherin expression in breast cancer in 276 total cases

Histologic Type Number (%)		Age (years) Mean ± SD	Nottingham Grade Number (%)			E-Cadherin Score Number (%)			
			I	II	III	3+	2+	1+	0
IDC	204 (73.9)	58.4 ± 15.0	29 (14.2)	83 (40.7)	92 (45.1)	199 (97.5)	4 (2.0)	0 (0.0)	1 (0.5)
IC	9 (3.3)	65.1 ± 13.5	0 (0)	8 (89)	1(11)	4 (44)	1 (11)	0 (0)	4 (44)
ILC	49 (17.8)	64.4 ± 13.0	46 (94)	3 (6)	0 (0)	2 (4)	3 (6)	0 (0)	44 (90)
PILC	10 (3.6)	58.2 ± 16.3	0 (0)	6 (60)	4 (40)	2 (20)	0 (0)	1 (10)	7 (70)
TLC	4 (1.4)	61.0 ± 9.1	2 (50)	2 (50)	0 (0)	—	—	—	—
Tubular		—	—	—	—	0 (0)	4 (80)	—	—
Lobular		—	—	—	—	—	—	2 (50)	2 (50)

IC: Invasive Carcinoma, IDC: Invasive Ductal Carcinoma, ILC: Invasive Lobular Carcinoma, PILC: Pleomorphic Invasive Lobular Carcinoma, TLC: Tubulolobular Carcinoma.

Table 2 Analysis of prognostic cancer biomarkers with loss of E-cadherin expression*

	All Cancers				Non Lobular Carcinomas			
Variable	No. (%)	EC+	EC–	*P* Value	No. (%)	EC+	EC–	*P* Value
Size				0.317				0.994
T1	153 (56.5)	126 (82.4)	27 (17.6)		126 (58.8)	124 (98.4)	2 (1.6)	
T2	92 (33.9)	70 (76)	22 (24)		67 (31.3)	65 (97)	2 (3)	
T3	13 (4.8)	8 (62)	5 (38)		9 (4.2)	8 (89)	1 (11)	
T4	13 (4.8)	12 (92)	1 (8)		12 (5.6)	12 (100)	0 (0)	
Grade				<0.001				0.114
I	61 (22.1)	34 (56)	27 (44)		31 (14.3)	31 (100)	0 (0)	
II	115 (41.7)	91 (79.1)	24 (20.9)		93 (42.9)	89 (96)	4 (4)	
III	100 (36.2)	94 (94.0)	6 (6.0)		93 (42.9)	92 (99)	1 (1)	
Node				0.167				0.798
N0	122 (59.8)	100 (82.0)	22 (18.0)		99 (62.7)	96 (97)	3 (3)	
N1	78 (38.2)	56 (72)	22 (28)		56 (35.4)	54 (96)	2 (4)	
N2	3 (1.5)	2 (67)	1 (33)		2 (1.3)	2 (100)	0 (0)	
N3	1 (0.5)	0 (0)	—		1 (0.6)	1 (100)	0 (0)	
ER				<0.001				0.783
Positive	208 (75.4)	154 (74.0)	54 (26.0)		153 (70.5)	149 (97.4)	4 (2.6)	
Negative	68 (24.6)	65 (96)	3 (4)		64 (29.5)	63 (98)	1 (2)	
PgR				0.127				0.598
Positive	187 (67.8)	144 (77.0)	43 (23.0)		141 (65.0)	139 (98.6)	2 (1.4)	
Negative	89 (32.2)	75 (84)	14 (16)		76 (35.0)	73 (96)	3 (4)	
HER-2/neu				0.765				0.635
Positive	47 (23.5)	41 (87)	6 (13)		43 (25.4)	41 (95)	2 (5)	
Negative	153 (76.5)	130 (85.0)	23 (15.0)		126 (74.6)	125 (99.2)	1 (0.5)	

EC: E-cadherin, ER: Estrogen Receptor, PgR: Progesterone Receptor, +: positive, –: negative. *: Data are given as number (percentage). The numbers of cases for size, grade, node, ER, PgR, and HER-2/neu for all carcinomas are 271, 276, 204, 276, 276, and 200, respectively, and for non lobular carcinomas, 214, 217, 158, 217, 217, and 169, respectively.

E-cadherin scoring used a 4-point scale adapted from [32]: negative = 0; weak and heterogeneous = 1+; mild or weak and homogeneous = 2+; moderate or strong and heterogeneous = 3+; intense or strong and homogeneous = 4+. The intensity of staining was scored from 0 – 3, where 0 = complete absence or negative; 1 = < 10% bright membrane expression; 2 = >10% but ≤ 50% membrane expression; and 3 = > 50% membrane expression.

Statistical Analysis

Statistical analyses were performed using SPSS-16 procedures (SPSS-16 Analytical Software Inc, Chicago, IL). Immunohistochemical staining scores were correlated with the histopathologic type, grade, nodal status, tumor size, hormone receptor status (ER and PgR), and HER-2/neu expression. The association between E-cadherin and tumor type was assessed with the $\chi 2$ test. Association with grade, nodal status, stage and HER-2/neu overexpression were assessed with the Spearman rank correlation coefficient. Associations with ER and PgR were assessed with the Cochran-Armitage trend tests, Kruskal-Wallis test, Wilcoxon rank sum test or the $\chi 2$ test, and confidence intervals. A 2-sided *P* value less than .05 was considered statistically significant.

Results

Patient demographics, histopathologic tumor subtypes, and tumor grade along with E-cadherin immunoreactivity are summarized in Table 1.

E-cadherin expression was seen in all but 1 case of IDC and special ductal types (203/204, 99.5%). As shown in Figure 1, E-cadherin expression was present in 100% of tumor cells in all positive cases, and the staining was 3+ in the majority (199 specimens) and 2+ in only 4 cases. The special types included 1 case of adenosquamous carcinoma, 3 cases of mucinous carcinoma, and 3 cases of

tubular carcinoma. Associated ductal carcinomas in situ (DCIS) was positive in 89 cases with 3+ E-cadherin immunoreactivity.

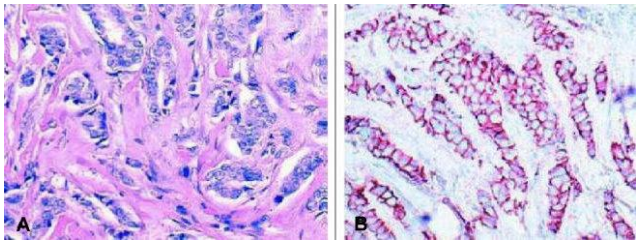

Fig. 1 Invasive ductal carcinoma: (A) H & E, (B) E-cadherin positive immunoreactivity. Magnification = 400X.

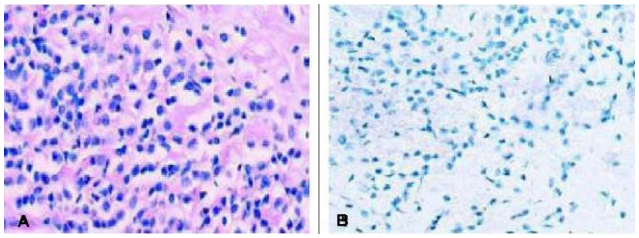

Fig. 2 Invasive lobular carcinoma: (A) H & E, (B) no E-cadherin immunoreactivity. Benign duct serves as a positive internal control. Magnification = 400X.

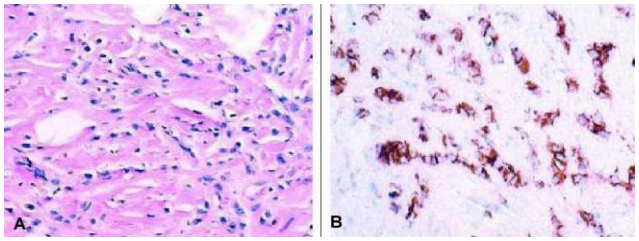

Fig. 3 Invasive lobular carcinoma: (A) H & E, (B) E-cadherin positive immunoreactivity. Magnification = 400X.

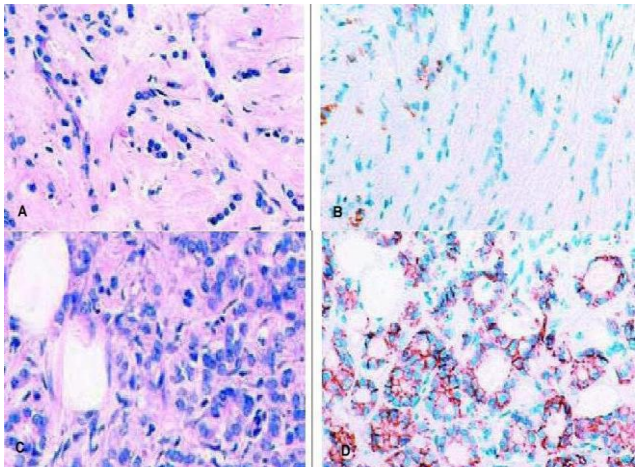

Fig. 4 Tubulolobular carcinoma with lobular component: (A) H & E, (B) E-cadherin negative immunoreactivity, Tubular component: (C) H & E, (D) E-cadherin positive immunoreactivity. Magnification = 400X.

Classic ILC was characterized by histopathology by strands of discohesive small to medium-sized tumor cells with mild to moderate cytologic atypia dispersed in a fibrous stroma. Of 49 ILC specimens with the classic histopathologic pattern, 44 (90%) showed complete loss of E-cadherin, as shown in Figure 2; 5 (10%) of typical histopathological ILC specimens showed complete membrane staining in 100% of tumor cells. Three of these E-cadherin-positive cases were well-differentiated nuclear grade I and II; 2 were moderately differentiated nuclear grade II according to the Nottingham grading system. Two cases of ILC had mixed alveolar and solid patterns, both of which were E-cadherin-negative. Twenty-five E-cadherin-negative conventional ILC cases also had E-cadherin-negative LCIS in the same slide. Two cases of E-cadherin-positive ILC had associated E-cadherin-positive LCIS, and 1 case had E-cadherin-positive DCIS.

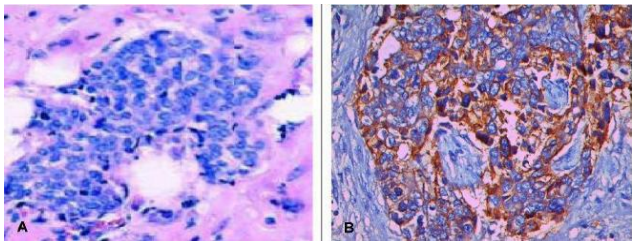

Fig. 5 Invasive carcinoma with lobular or ductal uncertainty: (A) H & E, (B) E-cadherin positive immunoreactivity. Magnification = 400X.

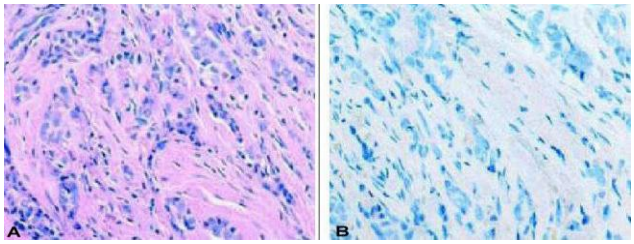

Fig. 6 Invasive carcinoma with lobular or ductal uncertainty: (A) H & E, (B) E-cadherin negative immunoreactivity. Magnification = 400X.

Pleomorphic ILC was characterized by histopathology by a growth pattern similar to classic ILC with greater cytologic atypia, pleomorphism, and discohesion. Of 10 cases of pleomorphic ILC, 8 (80%) showed loss of E-cadherin membrane staining in invasive and corresponding in-situ components, as shown in Figure 3. Two cases (20%) showed 3+ positive staining in 100% of tumor cells. One E-cadherin-positive pleomorphic ILC had E-cadherin positive LCIS.

The histopathology of TLC cases contained areas of classic ILC along with focal but distinct tubule formation. All cases of TLC exhibited a difference in E-cadherin expression between the tubules and the cords, with classic single-file pattern of ILC, as shown in Table 1. The tubules showed 2+ positive membranes staining, whereas the single-file invasive cords showed loss of E-cadherin, as shown in Figure 4. One case also had E-cadherin-positive DCIS, whereas E-cadherin-negative LCIS was present in 2 other cases.

Nine cases were designated as invasive carcinomas because of overlapping histopathologic features uncertain

for IDC or ILC. Of 9 cases, 5 (56%) showed positive E-cadherin staining in all tumor cells, as shown in Figure 5, whereas the remaining 4 cases (44%) were negative for E-cadherin staining, as shown in Figure 6.

Comparison of E-cadherin staining in IDC, ILC, and ILC variants revealed a highly significant difference between the groups (P<.001; Kruskal-Wallis test). Overall, negative staining of E-cadherin in ILC was specific for the diagnosis of ILC (specificity, 97.7%; negative predictive value, 96.8%; 95% confidence interval, 94.7-99.3). However, positive staining did not exclude the diagnosis of ILC (sensitivity, 88.1%; positive predictive value, 91.2%; 95% confidence interval, 77.1-95.1).

All invasive carcinoma associations between E-cadherin expression and tumor characteristics were assessed with the Wilcoxon rank sum test or the $\chi 2$ test. Various tumor variables (tumor size, nodal status, PgR status, and HER-2/neu status) did not reveal significant associations with loss of E-cadherin expression, as shown in Table 2.

However, loss of E-cadherin was significantly associated with tumor grade and ER status. The analysis was repeated after excluding all ILCs, on the basis that previous data have shown that ILCs are E-cadherin-negative irrespective of their grade, nodal status, size, or hormonal status. Complete loss of E-cadherin was seen in too few cases of IDC and special types to be of prognostic or predictive value, as shown in Table 2.

Discussion

E-cadherin is a cell adhesion molecule that is expressed in normal breast tissue and is useful as a phenotypic marker in breast cancer, with absence of its expression frequently observed in lobular type tumors. Reduced or impaired E-cadherin expression is associated with a reduced disease-free interval and overall survival and with other indicators of poor prognosis including a larger tumor size, higher histological grade, and development of distant metastasis and ER receptor negative tumors.

E-cadherin immunostaining can be used in finding patients with favorable outcomes among node-positive patients. The loss of E-cadherin expression is a very early change in lobular breast carcinogenesis and the normal protein plays a tumor-suppressive and invasion-suppressive role. E-cadherin staining can help differentiate between lobular carcinoma in situ (LCIS)/lobular carcinoma and ductal carcinoma in-situ (DCIS)/infiltrating duct carcinoma denoting the presence of DCIS or infiltrating duct carcinoma.

Foote and Stewart [33] used the term lobular carcinoma in situ for a special type of non-invasive carcinoma of the breast associated with a monotonous intralobular proliferation of cells. The concurrent invasive carcinoma with absence of tubule formation and single-file growth pattern was established as ILC [33, 34]. The distinctive histopathologic features of this special type of breast cancer described by Foote and Stewart [34] and Wheeler and Enterline [35] paved the way for identification of this tumor by histopathologist when the classic features are present.

Identification of solid, alveolar, tubulolobular, and pleomorphic variants [15, 17, 36-38] of ILC has added new dilemmas to the existing problem of distinguishing IDC of no special type with cord-like or trabecular patterns from ILC and its variants. Selective E-cadherin loss, now well recognized [17, 19, 22], validates ILC as a distinct entity and explains its histopathologic appearance [22] and distinctive growth patterns in metastases [23]. Although E-cadherin is emerging as an excellent biomarker to type breast cancers [17, 19, 22], the conflicting reports of E-cadherin loss as predictor of increased invasiveness, metastatic potential, and poor survival [24, 29] raise questions about its reliability for typing. Loss of E-cadherin alone cannot be a predictor of metastatic potential and negative outcome as E-cadherin is lost even in the pre-invasive stages of LCIS and atypical lobular hyperplasia. Furthermore, ILC is a slow-growing tumor that has been shown to have better survival than ductal carcinoma of no special type [13, 18].

As demonstrated in our study and in previous studies [23, 28], E-cadherin can help in the diagnosis of ILC. As in our study, complete E-cadherin loss has been reported in 86% to 100% of ILCs [19, 22], with most large studies reporting E-cadherin positivity in a small number of ILCs. All of these studies also show good membrane positivity for E-cadherin in all IDCs, including special types, even at the advancing front. Almost all of our cases of invasive and in situ breast cancers were strongly E-cadherin-positive (3+) or E-cadherin-negative (0). The exceptions were the few cases of TLC that showed 2+ staining in the tubules only and a very few high-grade cellular IDCs with apparent reduced expression of E-cadherin. Acs et al [19] also report similar “all or none” E-cadherin expression in the majority of their cases, including cases that were thought to have mixed or indeterminate patterns.

Berx et al [9] and Acs et al [19] observed variation in E-cadherin intensity in IDCs and in some cases of “ductolobular” carcinomas. TLC, first described by Fisher et al [39] as a rare variant of ILC, consists of a predominant ILC component with a diffuse infiltrative pattern and a component of variably defined small tubules [39]. All 4 of our TLCs fit this profile and had distinctive biphasic E-cadherin expression in 3 cases with no immunoreactivity in the ILC component and moderately positive immunoreactivity in the tubules.

Diagnostic difficulty occurs in some cases because IDC may show a dispersed growth pattern, including infiltration around benign ducts in a targeted manner similar to ILC [19]. Such cases were diffusely E-cadherin-positive in our study. Several authors have studied E-cadherin expression in ductolobular carcinomas

or carcinoma of indeterminate type with similar results. Of our 9 cases initially regarded as IC of uncertain type, 5 E-cadherin-positive cases seemed to be IDC with a dispersed growth pattern, whereas the 4 E-cadherin-negative tumors had morphologic features consistent with ILC. Thus, all of these cases could be classified further based on immunohistochemical expression of E-cadherin.

A category of mixed ductal lobular lesions is absent in our study because we were able to classify most lesions as ductal or lobular based on cytoarchitectural features. Most studies have observed retained E-cadherin expression in almost all IDCs but noted reduced expression, mainly associated with poor differentiation and high tumor grade [21, 22, 25, 27, 28].

Various studies have observed a correlation between reduced E-cadherin expression and lymph node status [28, 29] and ER and PgR status [27, 28]. Others have found no relationship to nodal or receptor status. To date, studies correlating E-cadherin expression with outcome are few. Some suggest that reduced E-cadherin expression may adversely affect overall and/or disease-free survival [24, 27] .

Siitonen et al [27] found reduced disease-free survival in association with reduced expression of E-cadherin. Charpin et al [24] found shorter overall survival in node-negative patients but did not see correlation with metastases or recurrence-free survival. Guriec et al [25] found reduced overall and disease-free survival. Acs et al [19] and Lipponen et al [30] demonstrated no correlation of E-cadherin expression with tumor size, grade, tubule formation, nuclear pleomorphism, mitotic activity, ER and PgR status, and HER-2/neu overexpression in invasive carcinomas.

Our findings were similar, with reduced expression being rare in non-lobular carcinomas, limited to a few high-grade IDCs. Moreover, as E-cadherin is retained in nearly all non-lobular invasive carcinomas, reduced expression is difficult to quantitative in a reproducible manner. Each reported study differs in evaluating the intensity, distribution, and quantitation of positive E-cadherin staining. Reduced staining and coarsely granular membrane staining seen in some very poorly differentiated IDCs in our study may represent a degenerative tumor effect.

Contrary to the observation that E-cadherin has an invasion-suppression role in vitro, E-cadherin is retained in the majority of non-lobular invasive carcinomas, including poorly differentiated tumors, and is lost in the majority of lobular breast cancer irrespective of stage, grade, hormone receptor status, HER- 2/neu expression, and nodal status. As previously pointed out [22, 26], invasiveness and metastatic potential of a tumor probably is dependent on a variety of currently identified and unidentified factors, rather than E-cadherin.

Conclusion

Loss of E-cadherin is a sensitive and relatively specific biomarker to confirm a diagnosis of ILC and its variants. A positive stain may not completely exclude the diagnosis ILC because E-cadherin expression may be retained in a minority of cases with characteristic ILC morphologic features. E-cadherin positivity clearly favors ductal differentiation in ambiguous cases.

Biphasic immunostaining confirms that TLC is a rare and distinct variant of ILC. Partial loss of E-cadherin in a minority of poorly differentiated IDCs is not of diagnostic significance. E-cadherin loss is rare in invasive non-lobular carcinomas and does not correlate with established prognostic variables when ILC is excluded.

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