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Short Communication

Correlation of E-Cadherin Expression with Differentiation Grade and Histological Type in Breast Carcinoma

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Recently, a correlation has been suggested between a loss of E-cadherin (E-CD) and increased invasiveness of neoplastic cells. In this study, E-CD expression in breast cancer was investigated using an affinity-purified antibody (ECCD-2) in an immunoenzymatic (avidin-biotin-alkaline phosphatase) test. Intensity and extension of E-CD immunoreactivity were evaluated in 61 breast carcinomas and correlated with their histological type and grade, nodal involvement, and hormonal receptor status. Histological types were infiltrating ductal carcinoma of no special type (n = 54) and infiltrating lobular carcinoma (n = 7). All infiltrating ductal carcinomas of no special type except two grade 3 carcinomas showed positive immunoreactivity that was variable among different cases. Grade 1 breast carcinomas (n = 10) showed greater immunoreactivity than grade 2 (n = 25) and grade 3 (n = 19) carcinomas. E-CD immunoreactivity correlated positively with the degree of tubular formation and inversely with the mitoses number. None of the infiltrating lobular carcinomas expressed E-CD in their infiltrating cells, whereas they showed only weak immunostains in areas of atypical lobular hyperplasia and lobular carcinoma in situ. These results indicate that E-CD expression correlates with histological type and grade in breast carcinomas. (Am J Pathol 1993, 142: 987–993)

Cadherins are a family of Ca^{2+}-dependent cell-cell adhesion receptors that play a major role during embryonic development and in the maintenance of adult tissue architecture.[1] Several types of cadherins have been identified, such as E-cadherin (E-CD) (uvomorulin, cell-CAM 120/180, L-CAM), P-CD, and N-CD (A-CAM). Although all subclasses share similar properties such as molecular weight, Ca^{2+} dependence, and protease sensitivity, each homophilic subclass is characterized by a unique tissue pattern distribution and selective interactions.[2] In adult organisms, E-CD is expressed in most normal epithelial tissues but is absent in lymphoid and connective tissues. Recently, E-CD expression has been demonstrated in a particular subset of sensory neurons.[3] E-CD immunoreactivity has been identified in carcinomas of different origins but not in mesenchymal or lymphoid neoplasias.[4,5] It has been suggested that the selective loss of E-CD can generate dedifferentiation and invasiveness in human carcinomas, supporting a role for E-CD as an invasion suppressor molecule.[6,7]

Breast carcinoma is an important cause of morbidity and mortality among women. A major research field is the identification of molecular events underlying invasiveness and metastatic progression of this neoplasia to develop prognostic indicators. In this re-

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port, we describe the results of our studies concerning the E-CD expression in breast carcinoma in an attempt to correlate these data with the usual clinicopathological features useful in the assessment of breast cancer prognosis.

Materials and Methods

Specimens

Breast tissue was obtained from 61 unselected mastectomy specimens submitted to the Department of Pathology, La Paz Hospital, Madrid. Neoplastic and nonneoplastic breast tissue samples were embedded in ornithine carbamoyltransferase compound (Miles Laboratory, Naperville, IL), snap-frozen in liquid nitrogen–cooled isopentane, and stored at −70 C. The remaining breast tissue and axillary lymph nodes were routinely fixed in 10% formalin for 24 hours and embedded in paraffin.

Immunohistochemical Staining

Immunostaining was performed as previously reported.[8] Briefly, 5 to 6-μ cryostat sections were immunostained by the avidin-biotin-alkaline phosphatase method. Nonspecific antibody binding was blocked with Tris buffer containing 2% goat serum. Tissue sections were incubated with the monoclonal antibody ECCD-2[9] (1/250), biotinylated sheep anti-rat IgG (1/200, Amersham International, Buckinghamshire, UK), and extravidin-alkaline-phosphatase complex (1/250, BioMakor, Rehovot, Israel). The alkaline phosphatase activity was developed using naphtyl ASMX phosphate as substrate and Fast-Red as the chromogen group (Sigma Chemical Co., St. Louis, MO). The sections were finally counterstained with Meyer hematoxylin.

The monoclonal antibody ECCD-2 was a generous gift of M. Takeichi (University of Kyoto, Kyoto, Japan). This rat monoclonal antibody against mouse cadherin also recognizes E-CD in the human mammary tumor cell line MCF-7.[10] Dilutions of ECCD-2 were carried out in 150 mmol/L NaCl, 10 mmol/L Hepes, pH 7.4, 10 mmol/L $CaCl_2$ (HMF-Ca buffer), containing 1% (wt/vol) bovine serum albumin. The secondary antibody was diluted in Tris buffer containing 1% bovine serum albumin. Normal mouse and human skin were used as positive control. These samples showed intense E-CD immunoreactivity along the cell membrane of keratinocytes. As negative control, we used rat anti-mouse IgG as the primary antibody. To evaluate tissue sample preservation, in all E-CD-negative specimens, serial sections were immunostained by monoclonal antibody class I major histocompatibility complex antigen (Dako, Copenhagen, Denmark). All these specimens showed a positive immunoreactivity of variable intensity for class I major histocompatibility complex in the epithelial and stromal cells.

Evaluation of Immunohistochemical Staining

A semi-quantitative estimation based on the staining intensity and relative abundance of E-CD immunoreactive cells was performed independently by two pathologists. The intensity was graded from 0 (equivalent to background staining of the acellular stroma) to +3 (intense stain equivalent to normal breast epithelium). The abundance of E-CD positive cells was graded from 0 to 4 by counting at least 100 tumor cells in areas of heterogeneous E-CD expression (0 = less than 5% of positive cells; 1 = 5 to 25%; 2 = 26 to 50%; 3 = 51 to 75%; 4 = 76 to 100%). With these data, a composite score was obtained by adding the values of the immunoreaction intensity and relative abundance. Preserved E-CD expression was estimated when the composite score was 6 or 7. Score 5 indicated moderately reduced E-CD expression, and scores 0 to 4 severely reduced E-CD expression. In six instances, there was a disagreement in the score assigned by the two pathologists, and the slides were reviewed by a third observer who did not know the nature of the antigen being tested and the hypothesis under investigation. A consensus based in the two most coincidental opinions was obtained.

Carcinoma histological typing was performed on formalin-fixed and paraffin-embedded samples. The combined histological grade (1, 2, and 3) of infiltrating ductal carcinomas was obtained according to Elston,[11] using the scale assigned to three features: tubular formation (1 to 3), nuclear atypia (1 to 3), and mitoses (1 to 3). The tumor size, lymph node status (0 versus any positive axillary lymph nodes), and the amount of estrogen and progesterone receptors of the tumor defined by the steroid binding assay (Dextran-coated charcoal analysis) were also evaluated. The χ^2 test was used to analyze the statistical significance between E-CD expression and the histological grade. The Kendall's test was used to evaluate the statistical correlations between E-CD expression and tubular formation, nuclear atypia, mitoses, tumoral size, nodal status, and estrogen and progesterone receptors.

Results

E-CD Expression in Normal Breast Tissue and Benign Breast Lesions

Lobular and ductal epithelium of normal breast tissue expressed E-CD in a regular array on lateral cell borders. A similar immunoreactivity pattern was observed in ductal hyperplasia.

E-CD Expression and Histological Type of Breast Carcinoma

Of the 61 samples analyzed, 54 cases were infiltrating ductal carcinomas of no special type (DCNST) and the remaining seven cases were infiltrating lobular carcinomas (ILC). The immunoreactivity scores estimated in the 54 DCNSTs are shown in Table 1. Twenty-seven DCNSTs had preserved E-CD expression (Figure 1, A and B, and Figure 2A), whereas in 27 cases it was reduced (Figure 1, C and D, and Figure 2B), including two cases with complete loss of E-CD (Figure 2C). The seven ILCs showed a marked alteration of E-CD expression. The areas of atypical lobular hyperplasia and lobular carcinoma *in situ* had decreased and heterogeneous E-CD immunoreactivity, whereas it was absent in both the pagetoid ductal spread and infiltrating cells in the stroma (Figure 3, A and B). In all evaluated ILCs, normal breast epithelium was present and showed preserved E-CD expression.

Correlation between E-CD Expression and Pathological Features in DCNSTs

Table 1 shows E-CD expression level in DCNSTs in relation to their histological grade. The frequency of tumors with reduced E-CD expression was significantly higher ($P = 0.0233$) in histological grade 2 and 3 breast carcinomas than in grade 1 tumors. A significant positive correlation was obtained when comparing E-CD expression with the tubular formation pattern, but a negative correlation resulted between the E-CD expression and mitoses. No correlation was observed with nuclear atypia, hormonal receptor levels (estrogen and progesterone), lymph node status, or tumor size.

Table 1. *E-CD Expression Compared with Histological Grade in Infiltrating Ductal Carcinoma of the Breast*

E-CD expression*	Histological grade		
	1	2	3
0–4	1	4	6
5	0	11	5
6	4	5	7
7	5	5	1
Total (54)	10	25	19

χ^2 test. $P = 0.0233$. Histological grade was obtained by assessing tubular formation, nuclear atypia, and mitoses according to Elston.[10]

* Composite score obtained by adding the intensity of immunoreaction (1 to 3) and relative abundance of positive cells (1 to 4). Normal breast epithelium had expression score 6 to 7. E-CD = E-cadherin.

Discussion

The E-CD expression in breast carcinoma has been mainly studied in cell lines,[5] and only two previous studies have analyzed the expression of this molecule in a reduced number of surgical specimens.[4,12] In these latter works, only a few examples of infiltrating ductal carcinomas have been evaluated. In the present study, we have analyzed for the first time the E-CD expression among different histological types of breast carcinomas. An interesting finding is the observation that lobular neoplasias are characterized by an important alteration in the E-CD expression. Decreased and heterogeneous expression was observed in lobular hyperplasia and in lobular carcinoma *in situ*, whereas all ILC infiltrating cells showed a complete loss of E-CD immunoreactivity. Similar results have been recently reported in ILC.[13] ILC is characterized by a diffuse infiltrating pattern of small and round, regular cells in single lines between collagen bundles.[14] Further studies analyzing a higher number of ILC and other adhesion molecules would be of interest to evaluate whether or not lack of E-CD expression could participate in the lack of cell adherence characteristic of ILC.

The present study confirms the previous observation in infiltrating ductal carcinomas that most tumors express this adhesion molecule, but some of them show quantitative abnormalities in their E-CD expression. Eidelman et al[4] studied five tumors and observed that all cases expressed E-CD. Shiozaki et al[12] have reported that nine of 20 cases (45%) displayed reduced E-CD expression in infiltrating ductal carcinomas. In concordance with these latter results, we have observed reduced E-CD expression in 27 of 54 cases studied (50%). However, these authors did not find any correlation between E-CD expression and the differentiation grade of breast carcinoma nor other clinicopathological features derived from the TNM classification. In contrast, we have found that E-CD expression correlates with the differentiation grade, the degree of

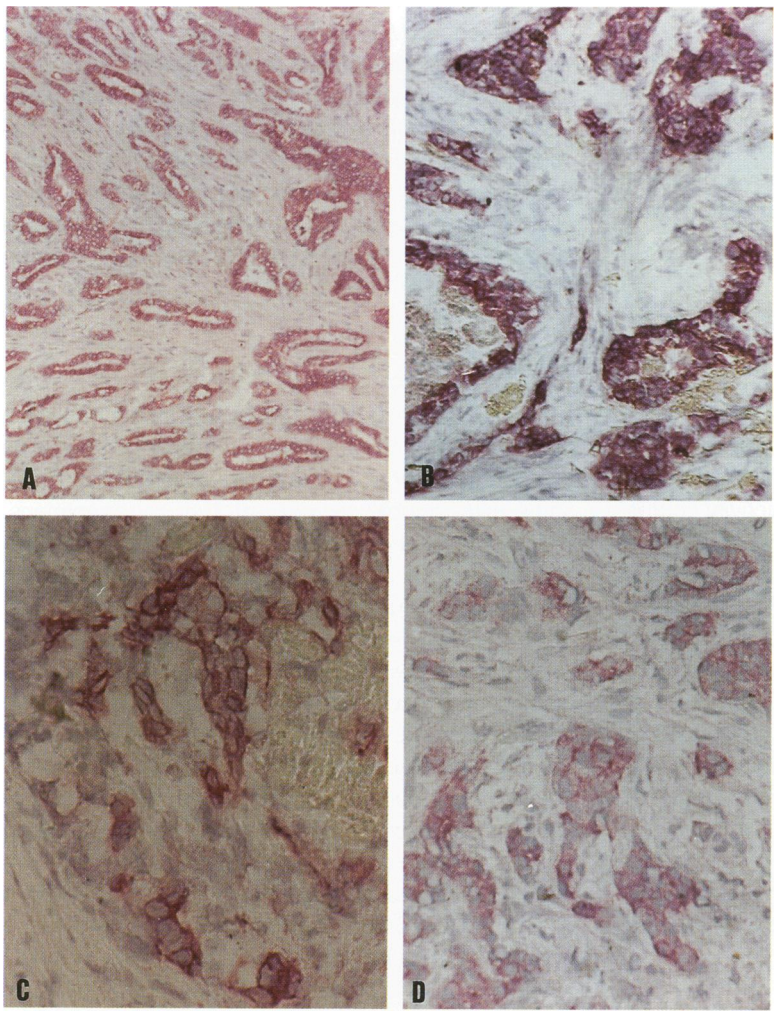

Figure 1. *Different patterns of E-CD expression in several infiltrating ductal carcinomas grade 1 and 2. E-CD immunoreactivity is preserved in a breast carcinoma grade 1 with prominent tubular formation* (**A**) *and in a grade 2 breast carcinoma with cohesive solid nests* (**B**). *Reduced E-CD expression in two grade 2 breast carcinomas with heterogeneous immunoreactivity among trabecular neoplastic cells* (**C** *and* **D**). *Note the weak immunostaining in* (**D**) (Original magnification ×40 in **A**, ×*63 in* **B**, *and* ×*60 in* **C** *and* **D**).

tubule formation, and mitoses. The discrepancy with both studies may be due in part to the difference in the number of cases analyzed. Our results in breast carcinomas are similar to those reported in gastric carcinoma.[12] In both types of neoplasias, highly differentiated tumors generally maintain strong and homogeneous E-CD expression, whereas in poorly differentiated carcinomas, E-CD-positive and negative cells can be mixed or, in extreme cases, expression can be virtually suppressed throughout the tumor, mainly in areas composed by poorly cohesive cells.

From studies with a variety of human cell lines, it has been suggested that E-CD represents an additional differentiation marker of human breast carcinoma cells. In addition, E-CD expression inversely correlates with *in vitro* invasiveness.[6] Cell lines derived from well-differentiated human carcinomas (which kept the epithelial phenotype in tissue cultures) generally express E-CD, whereas the molecule is not detected in most cell lines from poorly differentiated carcinomas. These cells show a fibroblastoid phenotype in tissue culture. Moreover, E-CD-negative cell lines were found to be invasive in collagen gels, whereas E-CD-positive lines generally did not.[6] Other experimental studies have suggested a reversible down-regulation of E-CD expression. Thus, *ras*-transformed Madin Darby Canine Kidney cells lose E-CD during tumor formation in the nude mouse, but re-expression of this adhesion molecule is observed in derived cell lines.[15]

It has been reported that *in vivo* E-CD expression inversely correlates with lymph node metastasis in squamous cell carcinomas of the head and neck[16] but not in gastric carcinomas.[17] Although we have found an inverse correlation between the differentiation grade of breast carcinomas and E-CD expression, numerous invasive high-grade tumors largely retain normal E-CD immunoreactivity, and no correlation was observed between E-CD expression and

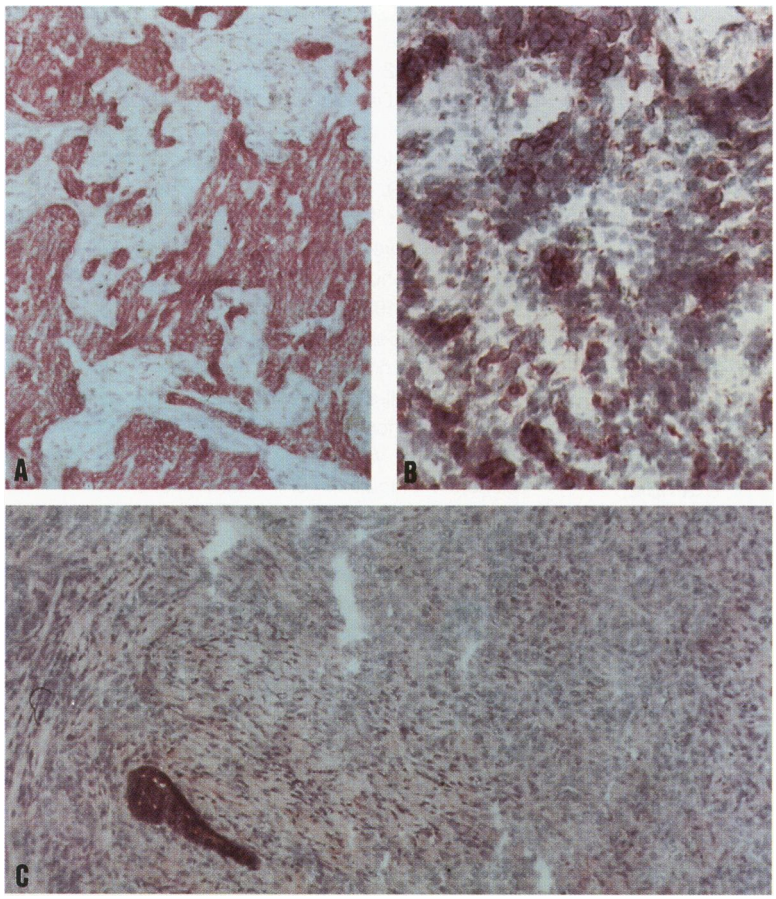

Figure 2. *Different patterns of E-CD expression in several infiltrating ductal carcinomas grade 3.* **A:** *E-CD immunoreactivity is preserved in this breast carcinoma with cohesive cells.* **B:** *Partial loss of E-CD expression.* **C:** *E-CD immunoreactivity is completely absent in a poorly differentiated breast carcinoma with noncohesive cells. Note E-CD expression in normal epithelium duct. (Original magnification ×40 in* **A** *and ×63 in* **B** *and* **C**).

nodal status. These observations could be explained by a temporary loss and posterior re-expression of E-CD or by biochemical suppression of the action of cadherins or their associated proteins. Thus, it has been reported that cadherin-mediated cell-cell adhesion is perturbed by v-*src* tyrosine phosphorylation of the cadherin-catenin system in metastatic fibroblast.[18] According to our results, we consider it unlikely that any single cell-surface alteration alone, such as loss of E-CD ex-

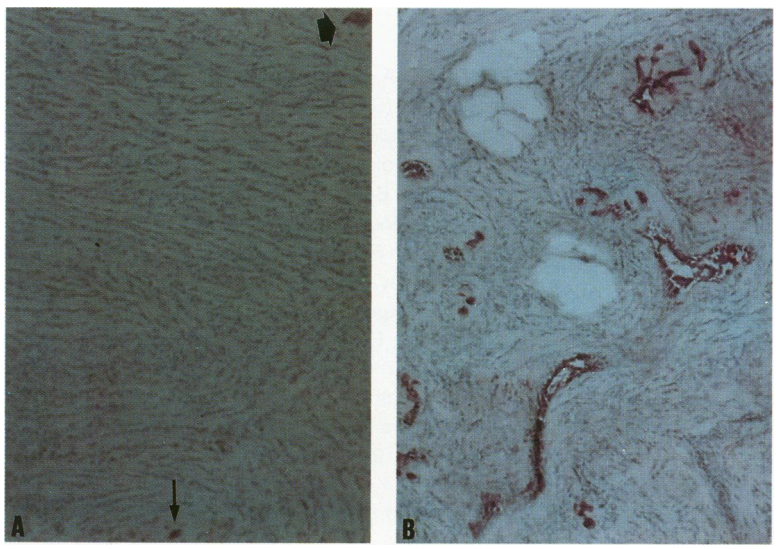

Figure 3. *E-CD expression in lobular carcinoma. E-CD immunoreactivity is negative in two examples of infiltrating lobular carcinoma with an Indian file pattern* **(A)** *and a targetoid pattern* **(B)** *of infiltration; note preserved immunoreactivity in normal ducts (arrows in* **A**). *(Original magnification ×16).*

pression, is responsible for the invasive and metastatic potential of neoplastic cells. Although invasiveness and metastasis can be enhanced by the down-regulation of E-CD expression, the alteration of other adhesion processes, such as cell-substrate adhesion,[19] or other genetic events such as low nm23 expression[20,21] might be necessary for the invasiveness and metastases development. Further studies are necessary to evaluate survival among breast carcinoma patients with different E-CD expression and to establish if this might be an independent predictor of survival in patients with this type of neoplasia, mainly in histological grade 2 and 3 carcinomas.

The estrogens seem to modulate the adhesive properties of breast carcinoma cells. For example, the expression of integrin adhesive receptors in breast carcinomas closely parallels the expression of estrogen receptors as judged immunohistochemically.[18] Moreover, it has been demonstrated that the breast carcinoma cell line MCF-7, which expresses estrogen receptors, also expresses E-CD,[6,10] whereas three dedifferentiated hormonal independent MDA-MB lines are E-CD-negative.[6] However, we could not find any correlation between the E-CD expression in ductal breast carcinoma and the estrogen and progesterone receptors expression assessed biochemically. Because this approach does not allow a morphological evaluation of both E-CD and hormonal receptor expression in the same cells, immunohistochemical analysis of estrogen and progesterone receptors is currently being performed in this DCNST series.

In short, results in the present series suggest that E-CD expression in breast carcinoma is more related to histological type and differentiation grade than with invasiveness and metastatic potential.

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