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Differential Loss of E-Cadherin Expression in Infiltrating Ductal and Lobular Breast Carcinomas

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The epithelial-specific cell-cell adhesion molecule E-cadherin was analyzed immunohistochemically on tissue sections of 89 human primary infiltrating breast carcinomas, using monoclonal antibodies 6F9 (for cryostat sections) and 5H9 (for cryostat and paraffin sections). The tumors included 41 well and moderately differentiated infiltrating ductal carcinomas (IDCs) most of which (78%) showed strong linear staining at the cell borders at a level, as high as luminal cells of normal mammary glands. The 26 poorly differentiated, more highly malignant IDCs examined also were all positive for E-cadherin, although a higher proportion of them (54%) showed reduced staining, which was heterogeneous and dotted over the cell borders. In contrast, 19 of 22 infiltrating lobular carcinomas (ILCs), which were either of the dispersed (classical), solid, or the mixed type, did not express E-cadherin, whereas three cases showed weak staining. **In situ** ***lesions of ILCs and pure lobular carcinoma*** **in situ** ***(four cases) were all E-cadherin negative, whereas intraductal carcinomas (11 cases) exhibited mostly strong staining. The results were confirmed by Western blotting. The data indicate that loss of E-cadherin expression is an early event in the formation of the lobular type of breast carcinomas. The absence of E-cadherin signifies a partial loss of epithelial differentiation and may account for the extended spread of lobular carcinoma*** **in situ** ***and the peculiar diffuse invasion mode of ILC. The generation of dedifferentiated IDCs can only in part be correlated with reduced expression of the intercellular adhesion molecule E-cadherin. Other factors are obviously also involved during invasion of this carcinoma type. (Am J Pathol 1993, 143:1731–1742)***

Breast cancer is one of the most frequent malignancies in women; in North America, every third female cancer patient suffers from this disease. Ductal and lobular carcinomas represent the main infiltrating breast tumor types, the latter being less frequent (5 to 10%).[1]

Several molecular components are possibly involved in the generation of breast carcinomas: mutations of the tumor suppressor genes p53 and RB have been detected in both cell lines and tissues.[2] The nm 23 gene product, which shows sequence similarity to nucleoside diphosphate kinases, is reduced in human melanomas, carcinomas, and sarcomas, and has antimetastatic potential on re-expression in tissue culture cells.[3] High nm 23 expression has been associated with good prognosis in patients with ductal breast carcinomas.[4] The HER-2/neu/c-*erb* B2 gene is amplified in 30% of breast and ovarian cancers. Amplification of neu is suggested to be of even higher prognostic value than the hormonal receptor status.[5,6] Expression of the estrogen receptor (ER) has been widely linked to both successful hormone therapy and retention of a more differentiated (grade 1 and 2) histological phenotype.[1,7] Epithelial-specific components such as cytokeratins have routinely been used for the identification of various carcinomas and their metastases. The expression of certain cell type-specific cytokeratins changes during the progression of breast and other carcinomas.[8]

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Our laboratory, among others, recently showed that the expression of E-cadherin, the major intercellular adhesion molecule of all epithelia, is important for the maintenance of a differentiated morphology and the prevention of invasiveness of MDCK epithelial[9] and some human carcinoma cell lines:[10,11] E-cadherin is well expressed in differentiated, noninvasive human carcinoma cell lines of various origins including breast, whereas dedifferentiated, invasive carcinoma cell lines lost E-cadherin expression. Forced expression of the E-cadherin cDNA in the MDA-MB-435S breast carcinoma cell line drastically reduced their invasive behavior *in vitro* indicating a causal role of E-cadherin for the suppression of invasiveness.[10] This causal role of E-cadherin has also been reported by others using different cell types.[12] E-cadherin expression was also studied in human squamous cell carcinomas of the head and neck: dedifferentiated, highly invasive primary lesions (100% of the cases) and 88% of lymph node metastases did not express E-cadherin messenger (m)RNA or protein.[11] Other laboratories screened lung, colon, stomach, liver, prostate, female genital tract, and a small number of infiltrating ductal breast carcinomas (IDCs); the results were less clear because some found that E-cadherin expression was generally not altered compared with the normal tissue,[13–16] whereas others reported on trends toward reduction or loss of expression in poorly differentiated cases.[17–21] Partially reduced expression of E-cadherin was found in squamous cell carcinomas of esophagus (73%), stomach adenocarcinomas (25%), and IDCs (45% of the cases).[22] A detailed analysis of E-cadherin expression of breast carcinomas linked to histological type and histopathological grading was previously lacking.

In this investigation, frozen tissue sections of 89 primary breast carcinomas were analyzed for E-cadherin expression using monoclonal antibodies described previously.[10] The tumor specimens consisted of 5 well, 36 moderately, and 26 poorly differentiated IDCs and of 22 infiltrating lobular carcinomas (ILCs). Moreover, six lymph node metastases of breast carcinomas were also included. Using monoclonal antibody 5H9, we also succeeded to detect E-cadherin in routinely formalin-fixed and paraffin-embedded tissue.

Materials and Methods

Tissues

Tissue material from 89 primary infiltrating breast carcinomas of untreated patients was included in this study. The histological types and grades are specified in Table 1. In addition, material from intraductal carcinomas (11 cases), lobular carcinoma *in situ* (LCIS; 4 cases), an ILC from a male, and lymph node metastases of IDCs (3 cases) and ILCs (3 cases) was analyzed. Normal breast tissue included in the surgical specimens was evaluated in 23 cases; normal resting breast tissue of tumor-free patients was obtained during reduction mammoplasties (n = 3, age 27 to 42 years) and during an autopsy (31-year-old patient, 4 hours postmortem). The tumor tissues were received within 30 minutes of surgical removal, were immediately snap-frozen in liquid nitrogen, and stored at −70°C. Routine histological examination was performed on parallel samples by formalin fixation and paraffin embedding and staining with hematoxylin-eosin and periodic acid-Schiff stains. In some cases, routine paraffin blocks were used for immunohistochemical staining using antibody 5H9. Conventional histological classification schemes were applied;[23,24] grading was performed according to ref. 25. Routine diagnosis also included determination of the estrogen receptor using the dextran-coated charcoal method (threshold value 20 fmol/mg protein) and/or immunohistochemical determination using the ER-ICA kit (Abbott Laboratories, Wiesbaden, Germany).

Immunohistochemistry

For immunohistochemistry, 5-μ thick cryostat sections were cut from the frozen tissue blocks. The following murine monoclonal antibodies were applied as primary antibodies: 1) antibody 6F9 specific for E-cadherin[10] (commercially available from Euro-Diagnostics, Apeldoorn, The Netherlands); 2) antibody 5H9 (an IgG 1), which is an E-cadherin-specific antibody derived from the same fusion experiment.[10] On Western blots of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) separated cell extracts and tryptic surface digests of human A-431 carcinoma cells,[10] antibody 5H9 reacted with both the 120 kd mature form and the 80 kd fragment of human E-cadherin (not shown). In an immunohistochemical analysis of frozen sections of human small intestine, antibody 5H9 stained the lateral cell borders of the intestinal epithelium with an accentuation of the subapical junctional complex (not shown); 3) antibody K_S 18.174 against cytokeratin 18[26] (commercially available from Progen, Heidelberg, Germany); 4) antibody AE1 against several cytokeratins including cytokeratin 19[27] (available from Progen); and 5) antibody VIM-9 against vimentin[26,28] (obtained from

Viramed, Martinsried, Germany). Immunohistochemical staining of 5-μ thick cryostat sections, which had been air-dried and fixed in acetone at −20°C for 10 minutes, was performed using the indirect immunoperoxidase method.[28] The primary antibodies were applied as hybridoma supernatants diluted up to 1:3 in phosphate-buffered saline (PBS). Routine paraffin sections were also stained for cytokeratins using antibody AE1 (including trypsinization).[26]

For monitoring E-cadherin on routine paraffin sections, 3- to 4-μ thick sections were mounted on glass slides coated with poly (L-lysine) and dried overnight at 58°C. After deparaffination and rehydration, the slides were immersed in 10 mM sodium citrate buffer (pH 6.0) and heated three to five times for 5 minutes in a microwave oven at 600 W.[29] After cooling to room temperature, endogenous peroxidase activity was blocked with 0.6% H_2O_2 in 40% methanol-PBS for 30 minutes. After pretreatment with 10% horse serum in PBS, the tissue sections were incubated with monoclonal antibody 5H9 (hybridoma supernatant, 1:10 in PBS; final IgG concentration 1 μg/ml) in a moist chamber at 37°C for 120 minutes. Bound antibody was detected using the avidin-biotin-complex (ABC) peroxidase method (ABC Elite Kit, Vector, Burlingame, CA). In most experiments (except the initial ones), nonspecific staining was blocked by adding 2% dried skim milk to the horse serum and all subsequent incubations.

Immunohistochemical staining was performed with 3,3′-diaminobenzidine and H_2O_2; for mild counterstaining, Mayer's hematoxylin solution was used.[28] In negative controls the primary antibody was replaced by PBS or an irrelevant monoclonal antibody. Normal glandular structures present in most breast carcinoma sections (at least once in each staining run) served as positive controls. With routinely fixed paraffin material, 5H9 staining was successful in most samples, with less than 10% of the tissues being poorly reactive (probably due to damage during fixation or tissue processing). Photomicrographs were taken using a Leitz Diaplan microscope on Agfapan 25 Professional film using two or three blue filters to enhance contrast.

Western Blot Analysis

For confirming antibody specificity, MCF-7 cells (kindly provided by Dr. W.W. Franke, German Cancer Research Center, Heidelberg) were scraped from the culture dishes using a rubber policeman, washed in Dulbecco's minimal essential medium, and lysed in SDS-PAGE sample buffer containing 2 mM $CaCl_2$ by brief sonication and heating to 95°C. The 20-μ thick cryostat sections of breast carcinomas were air-dried, and the tumor regions were carefully microdissected to avoid contamination by nontumorous epithelia.[26] After transfer to PBS, 2 mM $CaCl_2$, 0.4 mM phenylmethylsulfonyl fluoride, and centrifugation, the pellets were lysed as described above. After centrifugation at 13,000 *g* at room temperature, the cleared lysates were subjected to SDS-PAGE (8% polyacrylamide gels) and subsequent Western blotting.[26] Blots were incubated with antibody 5H9 at a 1:5 dilution.

Results

Monoclonal Antibodies Against E-Cadherin

We used two monoclonal antibodies, 6F9 and 5H9, for the immunohistochemical staining of E-cadherin on frozen sections of normal and malignant breast tissues. Most of the tissues were stained with both antibodies. Higher reactivity was revealed with antibody 5H9, ie, the staining intensity (in relation to the background) was higher and in some cases slightly higher percentages of immunostained tumor cells were recorded. Moreover, antibody 5H9 was also reactive on paraffin sections.

Expression of E-Cadherin in Normal Human Mammary Gland

Histologically normal glandular structures present in the breast carcinoma specimens and resting mammary gland tissue from patients without breast carcinomas were studied. By immunohistochemistry, the anti-E-cadherin antibodies strongly stained the intercellular borders of the luminal cells of both the interlobular ducts and the intralobular terminal ducts and ductules (Figure 1A and B). Myoepithelial cells of ducts and ductules showed much weaker reaction at cell-cell borders, which in paraffin sections was frequently resolved into fine dots (Figure 1B); in some specimens, the myoepithelial cells were negative.

Expression of E-Cadherin in Ductal Breast Carcinomas

Expression of E-cadherin was examined in 67 cases of IDCs (cryostat sections) that were grouped according to the histological grade (Table 1).

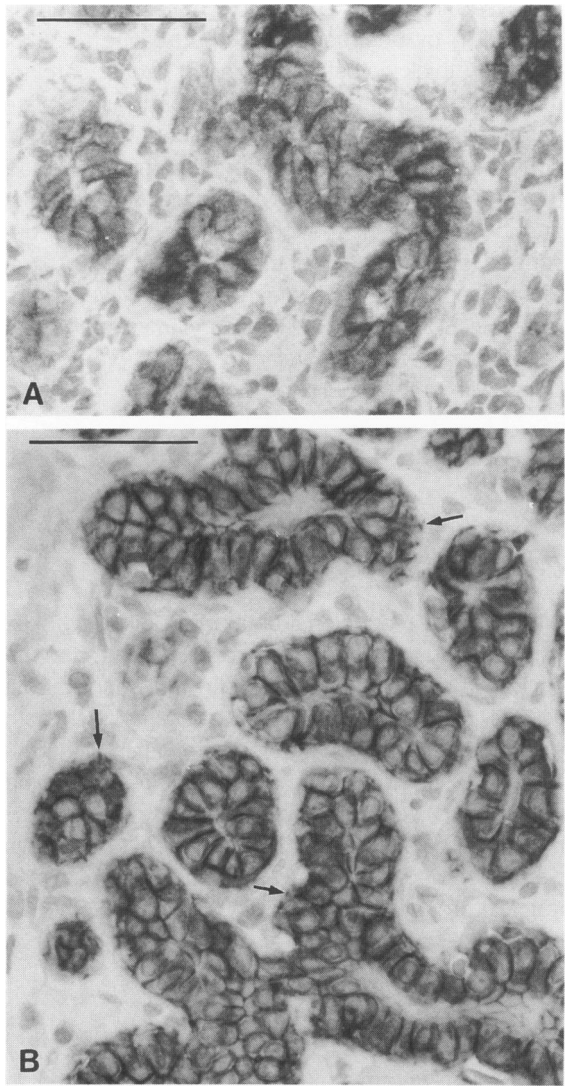

Figure 1. *Expression of E-cadherin in normal (resting) human mammary gland lobules as detected immunohistochemically on cryostat sections* (**A**) *(antibody 6F9; indirect immunoperoxidase staining) and on paraffin sections* (**B**) *(antibody 5H9; ABC-peroxidase method). The luminal cells of the terminal ductules exhibit linear intercellular immunoreactivity. Note the superior morphology of the paraffin section* (**B**) *also allowing resolution of the weak punctate staining of the myoepithelial cells at the basal side of the epithelium (arrows). Scale bar, 50 μ.*

E-cadherin was detected in all cases. Most of the G1/G2-tumors (78%) exhibited strong, predominantly linear staining of E-cadherin at the intercellular borders, which was present either throughout the tumor tissue or in the predominant portion (recorded as ++; see also Figure 2A_1 and A_2). In the remaining cases, reduced staining (recorded as +) was noted. The vast majority of the G1/G2-tumors (95%) were ER positive. Remarkably, approximately half of the poorly differentiated (G3) IDCs also showed strong and mostly linear staining for E-cadherin (see also Figures 2B and 4A and B), although these tumors exhibited low differentiation, as also reflected by the absence of detectable ER expression in 22 of 26 cases. Strong intercellular E-cadherin immunoreactivity was observed not only in more solid tumor cell formations (Figure 2B) but also in thin (one to two layered) tumor cell trabeculae that indicate a clearly invasive pattern (Figure 2C). Nevertheless, the G3-carcinomas differed from the G1/G2-group by the higher proportion of cases with reduced E-cadherin expression. In these cases cell border staining was generally weaker, discontinuous, mostly dotted, probably reflecting the decoration of adherens junctions,[30] and often some of the tumor cells even appeared essentially negative (Table 1; Figures 2D to F and 4C and D). IDCs with reduced E-cadherin still expressed the simple epithelial cytokeratins (Figure 2G), which in the normal mammary gland are located in the luminal epithelial cells. This indicates the maintenance of some epithelial characteristics and, in context with the morphological appearance of tight packaging, suggests remaining intercellular cohesion. Statistical analyses, using the χ^2 test, revealed good correlations ($P < 0.02$) between E-cadherin expression (strong vs reduced) and the degree of differentiation [well (G1)/moderately (G2) vs poorly (G3)] and between E-cadherin and ER expression.

An unusual case of breast carcinoma with focal spindle cell metaplasia (cryostat sections) revealed expression of E-cadherin only in the epithelial portion, whereas the spindle cell portion was negative (not shown). The spindle cell population of the tumor expressed predominantly vimentin, whereas simple epithelial cytokeratins were only focally present (not shown), indicating their predominantly mesenchymal phenotype.

Three cases of lymph node metastases from IDCs exhibited positive (strong or reduced) immunoreactivity for E-cadherin (Figure 3). Tumor cell clusters of IDCs within lymphatic vessels frequently showed positive intercellular E-cadherin staining (not shown).

In 24 randomly selected cases of IDCs, paraffin sections were processed for E-cadherin immunostaining using antibody 5H9. The results are summarized in Table 2. The qualitative and quantitative staining patterns were similar to those derived from cryostat sections. In fact, when the results obtained with frozen and paraffin material were compared with each other for the individual tumors, an identical score was noted for 22 tumors, whereas a deviating score was obtained for only two cases. This

Table 1. *E-Cadherin Expression in Human Primary Infiltrating Breast Carcinomas (Cryostat Sections)*

Type of infiltrating tumor	Histological grade	No. of cases and level of E-cadherin expression* (%)	ER expression (positive/negative cases)
Ductal	Well (G1) and moderately (G2) differentiated†	32 ++ (78.0)	29/1 (2 ND)
		9 + (22.0)	8/1
	Poorly differentiated (G3)	12 ++ (46.2)	2/10
		14 + (53.8)	2/12
Lobular‡	see§	3 (+) (13.6)	2/0 (1 ND)
		19 – (86.4)	14/1 (4 ND)

ND, not determined.

* Expression of E-cadherin was determined by indirect immunoperoxidase staining and was recorded as follows: ++ (strong), continuous linear intercellular staining (similar to the staining of normal luminal mammary epithelium) or strong very densely dotted intercellular staining present in the majority (>50%) of the tumor cells; + (reduced), markedly reduced and heterogeneous staining with predominantly finely dotted intercellular pattern (continuous linear staining if present restricted to <50% of tumor cells); (+), focal very sparse intercellular staining or weak cytoplasmic staining of tumor cells; – (absent), no reaction. In most of the cases recorded as ++, linear (or densely dotted) staining was present in >80% of the tumor cells; the remaining cells usually exhibited weaker dotted staining. The data of this table are based on the results obtained with antibody 5H9.

† The G1 (4 cases ++, 1 case +) and G2 (36 cases) primary ductal breast carcinomas were included in the same group because they were similar in both ER expression and in overall E-cadherin expression. This group could clearly be discriminated from the G3 carcinomas.

‡ 14 cases, classical form; 2 cases, solid variant; 6 cases, mixed form (classical and solid).

§ The standard grading method of Bloom and Richardson[25] is not applicable for ILC. It should, however, be mentioned that three cases (2 of solid variant, 1 of mixed form) showed increased nuclear atypia.

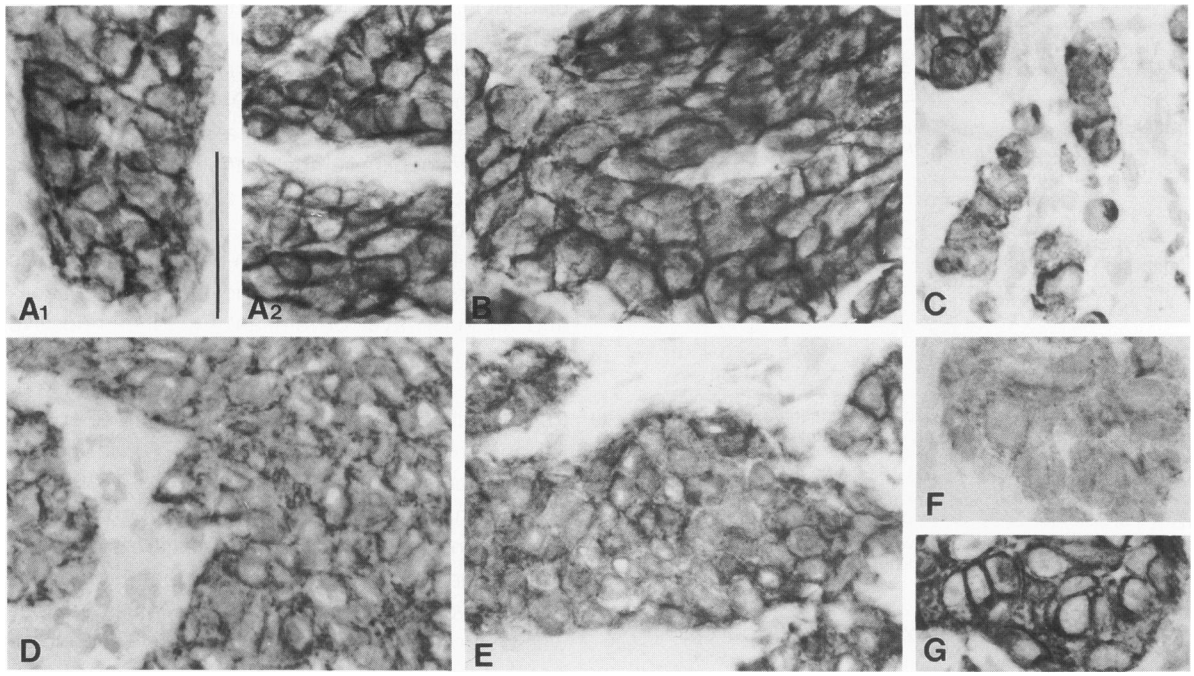

Figure 2. *Immunoperoxidase staining for E-cadherin of infiltrating ductal carcinomas of the breast (cryostat sections).* **A**, *moderately differentiated tumor showing strong (++, see Table 1) staining of the intercellular borders (A1, antibody 6F9; A2, antibody 5H9).* **B** *to* **F**, *poorly differentiated tumors with different intensities and patterns of E-cadherin expression (antibody 5H9) showing strong staining (++,* **B** *and* **C**) *including distinctly invasive portions* (**C**) *and reduced and heterogeneous staining (+;* **D** *to* **F**). *In* **G**, *the preserved expression of cytokeratins in a weakly E-cadherin-expressing case* (**F**) *is shown (antibody AE1; paraffin section). Scale bar, 50 μ.*

underlines the good reproducibility of the scoring, even between profoundly different immunohistochemical techniques, as well as the reliability of the paraffin section method. A considerable advantage of the latter method was that both morphology and resolution were superior. Examples of G3 IDCs with strong or reduced E-cadherin staining are illustrated in Figure 4A to D.

Intraductal portions of IDCs showed strong or occasionally reduced staining for E-cadherin (not shown). In addition, 11 cases of pure intraductal carcinomas (paraffin material) were studied (Table 3). In most of these cases, strong linear E-cadherin immunostaining of the intercellular borders was found that appeared linear or, in two of the cases, very densely dotted (recorded as ++; Table 3; Fig-

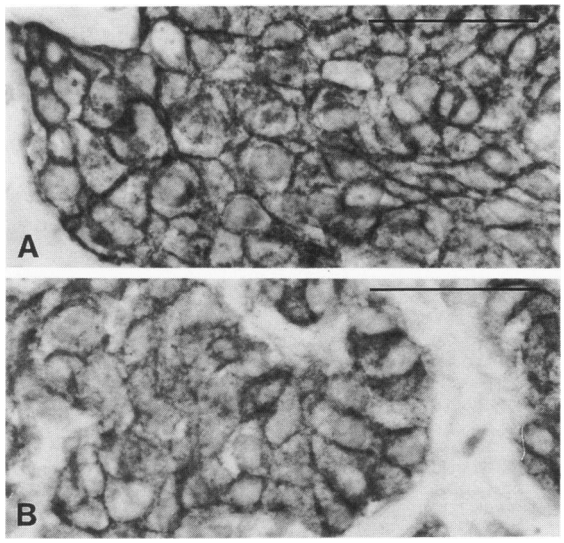

Figure 3. *Lymph node metastases of infiltrating ductal carcinomas (cryostat sections) showing strong (++; A) or reduced (+; B) immunoperoxidase staining for E-cadherin. Antibody 5H9 was used.* **A**, *poorly differentiated, ER-negative tumor,* **B**, *moderately differentiated, ER-positive tumor. Scale bar, 50 μ.*

Table 2. *E-Cadherin Immunostaining in Paraffin Sections of Human Primary Infiltrating Breast Carcinomas*

Type of infiltrating tumor	Histological grade	No. of cases and level of E-cadherin staining*
Ductal†	G1	3 ++
	G2	5 ++; 2 +
	G3	6 ++; 8 +
Lobular‡		3 (+); 11 −

* Paraffin sections were stained for E-cadherin as outlined in MATERIALS AND METHODS, using antibody 5H9. For definition of the scoring used, see footnote (*) in Table 1.

† All cases were primary tumors.

‡ All cases were primary tumors except for two cases (lymph node metastases, being negative for E-cadherin). 9 cases were classical form (2 of them being weakly positive), five cases were mixed form (classical and solid; 1 of them being weakly positive).

ure 5). Only one case exhibited reduced staining (recorded as +).

Expression of E-Cadherin in Lobular Breast Carcinomas

ILCs are characterized in their classical form by the highly microinvasive, dispersed histological pattern; in addition, different patterns such as the solid variant are observed.[24] The 22 cases of ILCs studied herein comprised classical, solid, and mixed forms. When ILCs were studied for E-cadherin expression (Table 1), they were, in contrast to the IDCs, mostly negative, as illustrated for frozen (Figure 6A to D) and paraffin material (Figure 6E and F; for paraffin section data see Table 2). For example, classical-type ILCs were almost always E-cadherin negative (Figure 6A and E). Entrapped normal ductal structures served as internal positive controls. Immunostaining for the simple epithelial cytokeratin 18 illustrated the highly dispersive growth pattern with scattered and single files of tumor cells (Figure 6B). Similarly, all tumors exhibiting the solid growth pattern were E-cadherin negative (Figure 6C and F), although the tumor cells formed solid cords and sheets as became evident by staining for cytokeratin 18 (Figure 6D). Most ILCs with mixed classical and solid pattern were also E-cadherin negative. The only exceptions were three cases of ILC (2 of classical form, 1 of mixed form) for which very sparse intercellular staining (Figure 6G) or a weak diffuse or punctate cytoplasmic immunoreaction (not shown) for E-cadherin was noted.

Interestingly, tumor cells of *in situ* portions of ILCs present in lobules and small ducts (eight cases), as well as the neoplastic cells of pure lobular carcinoma *in situ* (LCIS; four cases), were also negative for E-cadherin (Figure 7A to D). The lobular *in situ* aggregates were outlined by a peripheral layer of residual myoepithelial cells that frequently were weakly E-cadherin positive in a discontinuous pattern (Figure 7A). In ducts showing intramural spread of LCIS, the E-cadherin-negative tumor cells were interspersed between the myoepithelial cells and an inner layer of remnant E-cadherin-positive luminal cells (Figure 7B). Intraductal LCIS portions also were consistently negative for E-cadherin, contrasting with the positivity of residual luminal epithelial and myoepithelial cells (Figure 7C). In lobules partially occupied by LCIS cells, the tumor cell population was outlined by its negativity for E-cadherin, contrasting with the positive staining of the residual terminal ductule epithelium (Figure 7D). It is noteworthy that negativity of the *in situ* portions was also observed for the three weakly E-cadherin-positive ILC cases. Absence of E-cadherin expression was also noted in an ILC from a male (mixed form) and in three cases of lymph node metastases derived from ILCs (not shown).

Western Blot Analyses

To confirm the immunohistochemical data on E-cadherin expression, we performed Western blot analyses of total cell and tissue protein extracts, using monoclonal antibody 5H9. A single band of approximately 120 kD, corresponding to intact E-cadherin[10,11] was recognized in control MCF-7 cells. A band of the same size was present in

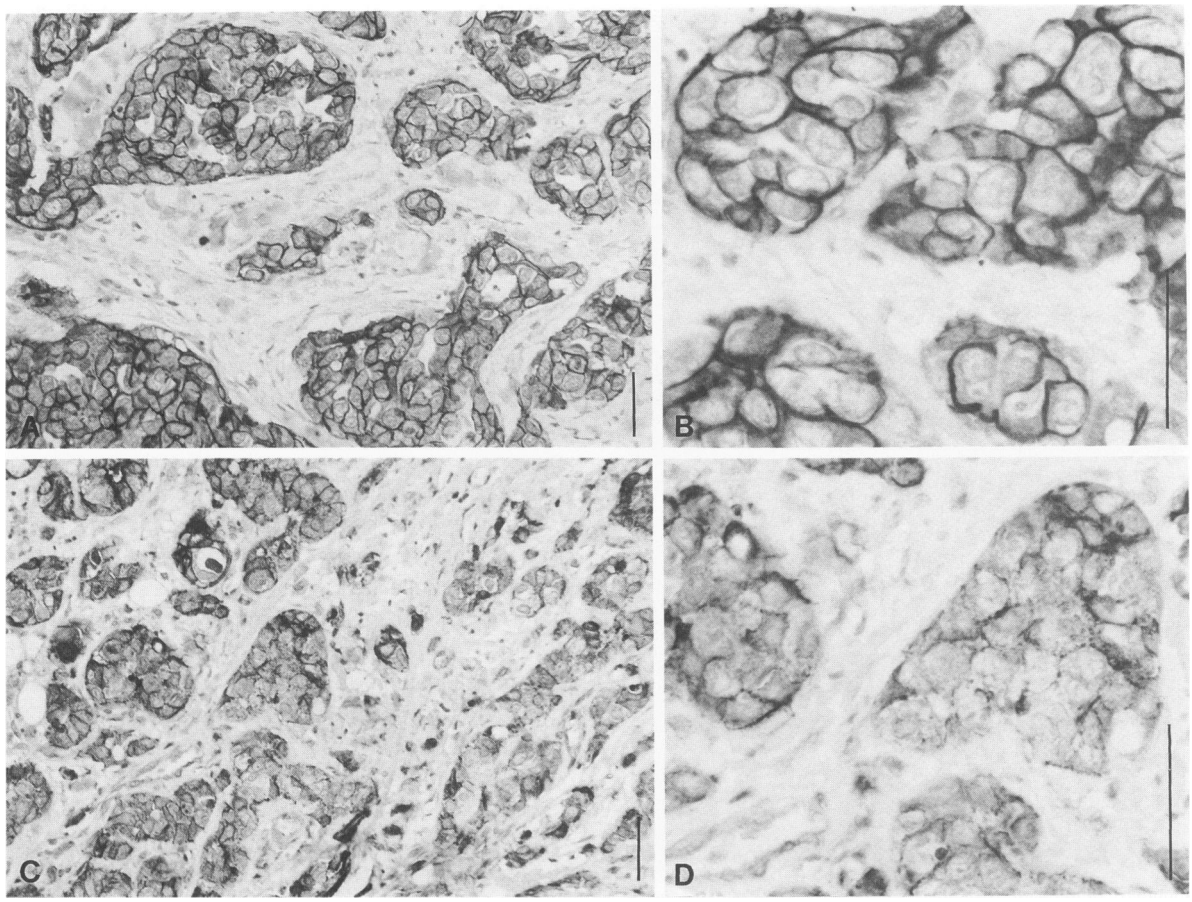

Figure 4. *Paraffin sections of poorly differentiated (G3) infiltrating ductal breast carcinomas (ER negative) immunostained for E-cadherin using antibody 5H9 (ABC peroxidase method). The case shown in* **A** *and* **B** *exhibits strong linear staining, whereas the tumor illustrated in* **C** *and* **D** *shows reduced, heterogeneous staining with a clearly dotted pattern. Scale bars, 50 μ.*

Table 3. *E-Cadherin Expression in Pure* In Situ *Carcinomas of the Breast*

Histology	No. of cases and level of E-cadherin staining*
Intraductal carcinoma†	10 ++; 1 +
Lobular carcinoma *in situ*	4 –

* Paraffin sections were immunostained for E-cadherin as outlined in MATERIALS AND METHODS, using antibody 5H9. For definition of the scoring used, see footnote (*) in Table 1.

† The histological patterns were as follows: 2 cases, solid; 1 case, solid and cribriform; 2 cases, comedo; 2 cases, comedo and cribriform; 2 cases, cribriform; 1 case, papillary and cribriform; 1 case, papillary. The case scored as + contained comedo and cribriform structures.

preparations from IDCs, whereas in ILC preparations this protein was absent (Figure 8).

Discussion

In this study we have analyzed the expression of the intercellular adhesion molecule E-cadherin in sections of normal human mammary glands and breast carcinomas. We used two different monoclonal antibodies, the previously characterized 6F9[10] and the newly introduced 5H9; the latter proved to be an excellent reagent to detect E-cadherin in routinely formalin-fixed, paraffin-embedded material. We found that E-cadherin is well expressed at the borders of the epithelial cells in normal glands, in most noninvasive intraductal carcinomas, and also in most of the well or moderately differentiated IDCs, which are generally only moderately aggressive. Half of the poorly differentiated and highly invasive IDCs showed considerably reduced E-cadherin staining. Remarkably, a total loss of E-cadherin expression was found in most ILCs and in LCIS. This expression mode was evident from both cryostat and paraffin sections and was also confirmed by Western blotting. In contrast, the simple epithelial cytokeratins 18 and 19, which are well expressed in the luminal epithelia of normal mammary glands, retained high level of expression in the IDCs (Figure 2G) and ILCs (Figure 6B and D).

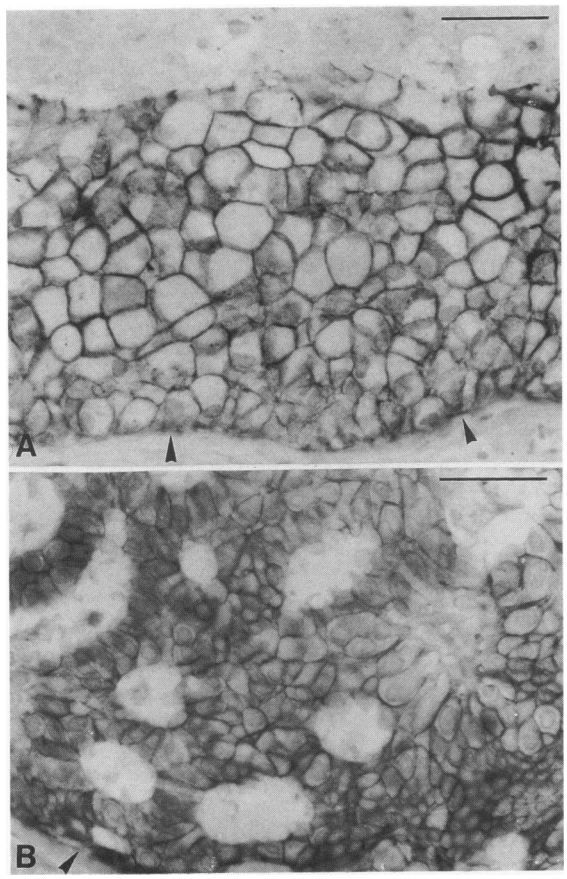

Figure 5. *Intraductal carcinomas immunostained for E-cadherin (paraffin sections; antibody 5H9; ABC peroxidase method). Strong linear intercellular staining is seen both in comedo type* (**A**; *necrotic debris on top*) *as well as in cribriform type* (**B**). *Arrowheads denote the ductal basement membrane. Scale bars, 50 μ.*

Our finding of a clear-cut difference in the E-cadherin expression between IDCs and ILCs suggests a fundamental difference in the cellular mode of invasion in these two breast cancer types. This has already been suggested by morphological observations[24] but is now confirmed by the examination of a defined molecular component. At the invasion front of IDCs, E-cadherin expression is often retained (Figure 2C). This indicates that invasive tumor cells of IDCs still form true cohesive, though often thin and threadlike, epithelial cell units whose intercellular connections are maintained not only by desmosomes[31] but also by adherens junctions containing E-cadherin molecules. Whether E-cadherin at these locations is functional cannot be decided by our analysis. In contrast, ILCs typically invade the connective tissue in a more diffuse manner, thus showing unsharp tumor borders, which results in a high rate of local recurrencies. In this tumor type, the absence of E-cadherin may now be associated with the peculiar diffuse invasion mode. It should be noted, however, that the expression of other differentiation markers such as ER and mucus is retained[32] indicating that ILC cells are not completely dedifferentiated. It is interesting that in the precursor lesion of ILC, LCIS, E-cadherin is already absent. This was observed not only in fully developed LCIS lesions, which obliterate lobular terminal ductules and larger ducts, but also in early stages of infestation such as intramural spread within the ductal epithelium and partial involvement of lobules (Figure 7). The extended intraductal spread and multicentricity of this *in situ* tumor may be a direct consequence of a molecular defect in E-cadherin expression because the tumor cells may thus easily dissociate and freely move along the ductal system. It should be noted, however, that the lack of E-cadherin in LCIS cells does not confer the ability to penetrate the acinar/ductal basement membrane per se because LCIS may persist *in situ* for many years. Thus, in the lobular type of breast cancer, loss of E-cadherin is not a key event for the initial phase of invasion. In conclusion, the common lack of E-cadherin as a distinctive feature of both LCIS and ILCs supports the hypothesis that both are closely related and constitute one distinct tumor entity.

Concerning IDCs, we found a correlation between reduced E-cadherin expression and poor degree of differentiation. The failure of others to find this correlation[22] may be due to the lower number of cases analyzed (20 vs 67). In this context, it should be stressed that in this study all IDCs, including poorly differentiated cases, retained at least some expression of E-cadherin; this is clearly different from other tumor types such as colonic and prostatic adenocarcinomas and head and neck squamous cell carcinomas, which when poorly differentiated reportedly often are completely E-cadherin negative.[11,18,19] The groups of G3-IDCs that show retained or diminished expression of E-cadherin did not reveal further differences concerning morphology or ER content. Thus, one would currently assign a similar prognosis to these two subtypes of tumors. Future work is therefore required to see whether the expression of E-cadherin in this tumor type can be correlated with patient prognosis. As a further potential application, E-cadherin could also be used as a histodiagnostic marker to discriminate between IDCs and ILCs and between the ductal and lobular type of *in situ* carcinoma, as well as to elucidate the true nature of so-called mixed differentiated carcinomas.[1,32,33]

The lymph node metastases of IDCs tested, as well as tumor cell clusters of lymphangiosis carcino-

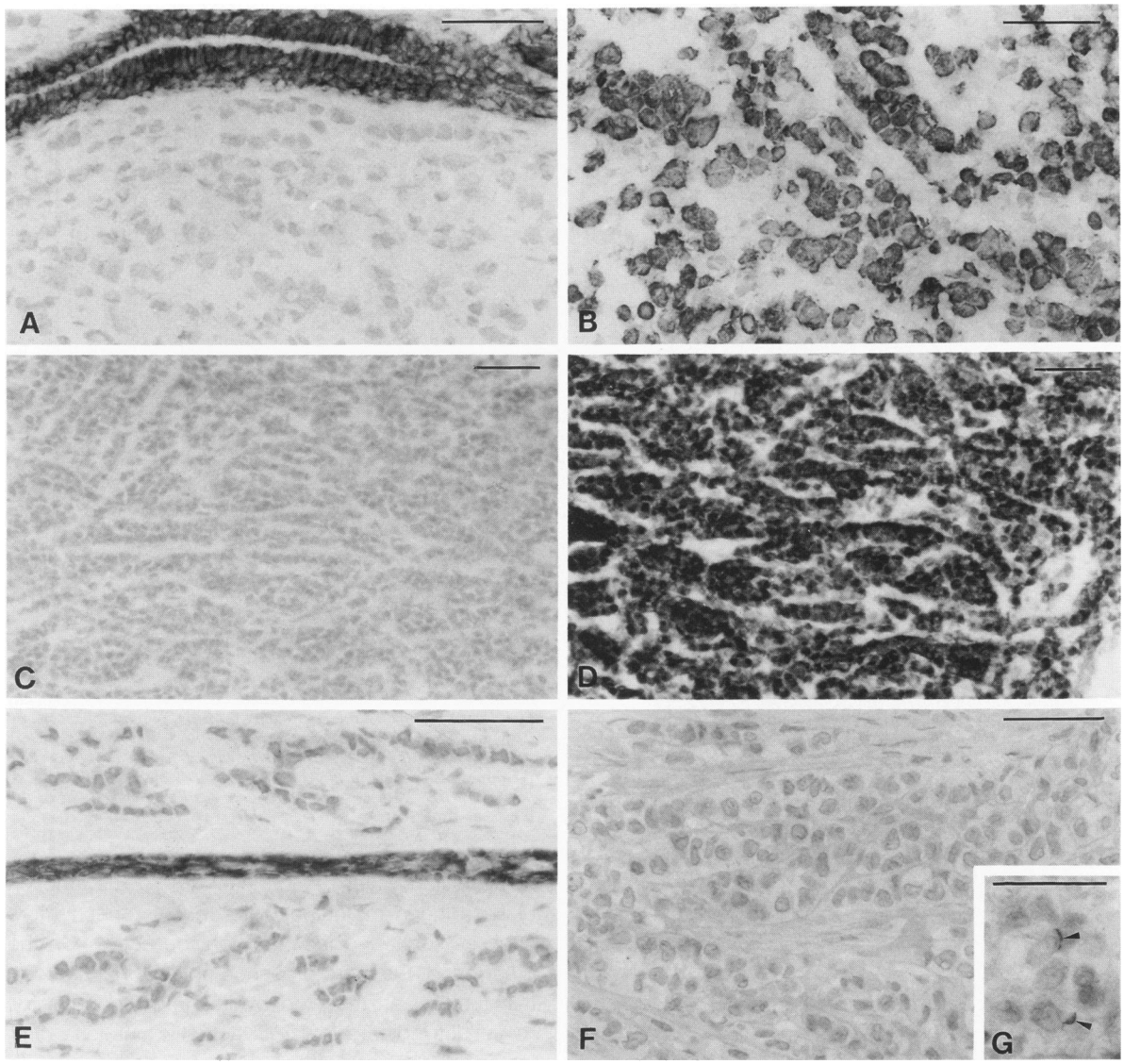

Figure 6. *Immunoperoxidase staining for E-cadherin (antibody 5H9) and cytokeratin of infiltrating lobular breast carcinomas (A to D, cryostat sections; E to G, paraffin sections). A, classical (highly dispersive) form and C, solid portion of a mixed variant. Both were negative for E-cadherin expression, whereas a residual normal glandular duct (A, top) was positive. B and D, same cases as A and C stained for cytokeratin 18 (antibody K_S 18.174). E and F, paraffin sections showing similar results, ie, negative E-cadherin immunostaining of both classical type (E) and solid type (F), whereas a compressed residual duct is strongly stained (E). Exceptionally, very sparse and focal staining can be seen in the case shown in G (arrowheads denote positive cell borders). Scale bars, 50 μ.*

matosa, showed expression of E-cadherin, whereas node metastases of head and neck carcinomas were reported to be largely negative.[11] This shows that the presence of E-cadherin in the gross majority of tumor cells of ICDs is principally compatible with detachment of cells or small cell groups during metastasis formation. On the other hand, the tendency toward reduced expression of E-cadherin in G3 IDCs indicates that loss of E-cadherin expression might be of importance in certain cases. Clearly, more examples need to be analyzed before the function of E-cadherin in metastasis formation of breast carcinomas is definitely established.

The question arises which factors might promote invasiveness of IDCs with high E-cadherin expression. Recently, cell motility factors like scatter factor/hepatocyte growth factor and acidic fibroblast growth factor have been described, which induce invasiveness of diverse epithelial cell lines *in vitro* without changing E-cadherin expression.[34–36] Such components might also be important for the invasion of E-cadherin-positive IDCs. Undifferentiated, scattered-type gastric carcinomas are comparable in growth and invasion with ILCs. In contrast to ILCs, the great majority of such tumors had preserved expression of E-cadherin, despite the single

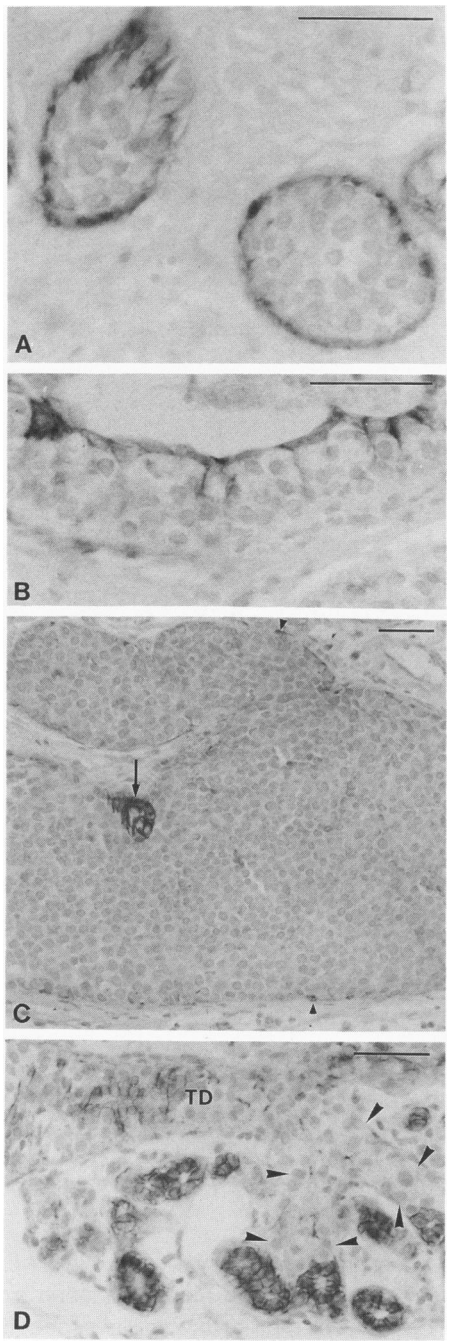

Figure 7. *Immunostaining of LCIS for E-cadherin on paraffin sections using antibody 5H9 (ABC peroxidase method).* **A**, *E-cadherin-negative tumor cells filling lobular terminal ductules. Note the positive remnant myoepithelial layer.* **B**, *intramural spread of E-cadherin-negative tumor cells within the epithelium of an interlobular duct. The residual luminal cell layer is positive.* **C**, *intraductal growth of LCIS being E-cadherin negative. Note strongly immunoreactive residual luminal cells (arrow) as well as weak staining of some myoepithelial cells (arrowheads).* **D**, *partial involvement of a lobule by E-cadherin-negative tumor cells (arrowheads). Linear E-cadherin immunostaining is seen in tubular remnants of the ductular epithelium at the distal ends of the lobule. TD, terminal duct. Scale bars, 50 μ.*

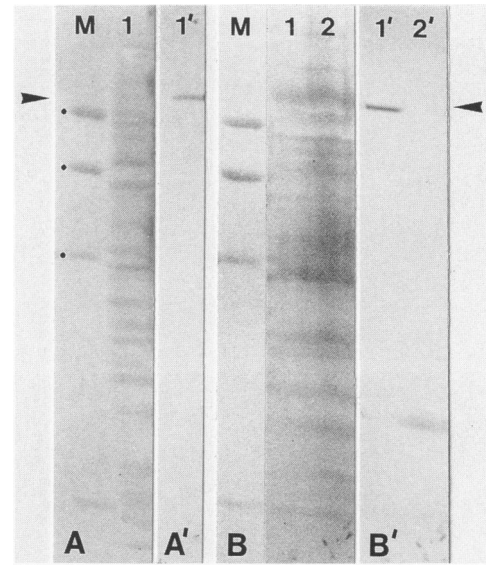

Figure 8. *Western blot analysis of total protein extracts of MCF-7 cells and breast carcinoma tissue using antibody 5H9.* **A**, *Ponceau-S red staining of transferred proteins after SDS-PAGE. Lane M, marker polypeptides (dots denote from top to bottom: β-galactosidase, phosphorylase b, bovine serum albumin); lane 1, MCF-7 cell proteins.* **B′**, *corresponding Western blot reaction of the same nitrocellulose membrane as shown in* **A**. **B**, *Ponceau-S red staining: lane M, marker polypeptides; lane 1, proteins from IDC (G2; immunohistochemically E-cadherin positive); lane 2, proteins from ILC (immunohistochemically E-cadherin negative).* **B′**, *corresponding Western blot reaction. An immunoreactive 120 kd (arrowheads) band representing E-cadherin is seen in MCF-7 cells (***A′**, *lane 1′) and IDC (***B′**, *lane 1′) but not in ILC (lane 2′; the weak low molecular weight band in this lane is nonspecific).*

cell invasion pattern.[16] In these tumors, the function of E-cadherin might be disturbed or other factors such as scattering factors might be active. On the other hand, the mere presence of E-cadherin in certain tumors, as revealed by immunocytochemistry, might not indicate that it is actually functional. For instance, certain mutations in the E-cadherin gene or changes in the E-cadherin-associated cytoplasmic proteins, the catenins,[37] might just weaken the adhesive capability of the molecule.

In human hepatocellular, prostate, and breast carcinomas, allele loss has been detected on chromosome 16 q 22.1 to 23.2[38–40] where the E-cadherin gene is located.[41] Thus, decline of E-cadherin expression in some breast cancers could be due to a direct mutation in the E-cadherin gene.[18] Alternatively, the promoter of the E-cadherin gene could be repressed because regulatory elements in this promoter have actually been found to be active in differentiated noninvasive and inactive in dedifferentiated invasive breast carcinoma cell lines.[42] In conclusion, the examination of the cell adhesion molecule E-cadherin might prove important not only for understanding the basic mechanisms involved in the progression of malignant epi-

thelial tumors but might also be used as a histological marker for refinement of pathological diagnosis.

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Note Added in Proof

After submission of our manuscript, other articles on E-cadherin in breast carcinomas, using different monoclonal antibodies, have appeared.[43–45] The present results (see also[46]) are generally in agreement with those reported by the other groups. Correlations between E-cadherin immunostaining and the degree of differentiation of IDCs have also been found by two of these groups.[44,45] It should be noted that the findings of positive (albeit decreased) immunoreactivity for E-cadherin in LCIS areas,[44] and (uniformly or heterogeneously) positive E-cadherin staining in 3 of 4 cases of ILCs studied[45] are not in line with the present data and those of ref.[43].

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