Expression of E-Cadherin Cell Adhesion Molecules in Human Breast Cancer Tissues and Its Relationship to Metastasis

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ABSTRACT

E-cadherin (E-cad) is a subclass of the cadherin family that plays a major role in the maintenance of intercellular junctions in epithelial tissues. In order to explore the correlation between the expression of E-cad and cancer invasion and metastasis in vivo, we performed an immunohistochemical examination for E-cad expression in 120 patients with breast cancer using our specific anti-E-cad monoclonal antibody.

In noncancerous epithelial cells, E-cad was strongly expressed on cell-cell boundaries, whereas various staining patterns were observed in tumors. Of these 120 tumors, 56 (47%) showed Pr type expression of E-cad, and 64 (53%) showed Rd type or negative expression. We found significant correlations between E-cad expression and clinical-pathological features. The frequency of Rd type was significantly higher in invasive ductal carcinomas (58%, 56 of 97) and poorly differentiated carcinomas (84%, 21 of 25) than in noninvasive and well-differentiated carcinomas.

Furthermore, a high frequency of Rd type was detected in the following advanced tumors: T3, T4 tumors, 71% (22 of 31); tumors with extensive lymph node metastasis, 74% (29 of 39); and tumors with distant metastasis, 86% (19 of 22). These values were significantly higher compared with their counterparts.

The expression of epidermal growth factor receptor tended to be positive in E-cad-positive tumors. However, no significant relationship was seen among E-cad expression, menopausal status, hormone receptor status, and DNA ploidy pattern. These results suggest that the reduction of E-cad expression may play an important role in invasion and metastasis of human breast cancer.

INTRODUCTION

The process of cancer invasion and metastasis consists of a complex series of sequential steps, involving specific tumor cells and host properties (1, 2). Detachment of tumor cells from the primary lesion is assumed to be the initial and important step in the metastatic process (3, 4). In 1961, Coman (5) described tumor cells as more easily separated from a solid tumor mass than their counterpart, normal cells from surrounding tissue and their detachment as regulated by the property of tumor cell "adhésiveness." However, the molecular basis of the mutual adhesiveness of cancer cells has not been clarified in vivo, and it is difficult to estimate the actual strength of intercellular connection from the expression of a single adhesive molecule.

Cadherins are the family of functionally related transmembrane glycoproteins responsible for the Ca2+-dependent cell-cell adhesion mechanism that is crucial for the mutual association of vertebrate cells (6). They were identified by preparing antibodies capable of blocking intercellular adhesion between mouse teratocarcinoma cells (7). Also, they were proved to represent a gene family completely different from those of integrin and immunoglobulin superfamilies using complementary DNA cloning (8). Cadherins bind cells tightly by homophilic interaction in the presence of Ca2+ (9), and the inactivation of other adhesion systems has little effect on cell-cell adhesion when cadherins are functioning (10, 11). However, treatment of cell layers, expressing cadherins, with anti-cadherin antibodies induces the dispersion of cells (12, 13). These findings indicate that cadherins play a major role in intercellular physical adhesion and that the number of cadherin molecules expressed in a cell directly affects its adhesiveness.

Cadherins can be subclassified into E-cad (7, 14), neural type cadherin (15), placenta type cadherin (16), L-CAM, and others (17, 18), and the amino acid sequences of these subclasses are highly homologous (16). Each type of cadherin subclass specifically interacts with the identical type (9) and is thought to play a key role in the morphogenesis with their unique spatiotemporal expression pattern during embryogenesis (15, 19). E-cad molecules, which are distributed mainly in epithelial tissues, have been demonstrated to be responsible for organogenesis and morphogenesis of these tissues (20). They have been given different names by various investigators, e.g., Arc-1 (21), uvomorulin (22), L-CAM (17), and cell-CAM 120/80 (23).

From these backgrounds, it is believed that the suppression of E-cad activity might trigger the release of tumor cells from the primary lesion in cancerous tissue. In 1989, J. Behrens et al. (24), using Madin-Darby canine kidney cells transformed with Harvey and Moloney sarcoma viruses, demonstrated that inhibition of cadherins by antibodies promotes cell invasion in model systems in vitro (24). Furthermore, recent studies with transfected cell lines have shown that selective loss of E-cadherin expression can generate dedifferentiation and invasiveness of human carcinoma cells (25).

We recently demonstrated the existence of abnormal E-cad expression in human cancerous tissues (26) and a significant relationship between E-cad expression and histological grade or invasiveness in gastric cancer (27). Schipper et al. (28) also suggested that down-regulation of E-cad expression is associated with dedifferentiation and lymph node metastasis of squamous cell carcinomas of the head and neck (28). However, the role of E-cad molecules in human cancer has not been clarified by a clinicopathological study with statistical analysis. In order to reveal the correlation of E-cad molecules with invasion and metastasis of human cancer cells in vivo, we studied E-cad expression in surgically resected human breast cancer tissues and metastatic lymph nodes by means of immunohistochemical staining.

We also investigated the relationship of the expression of E-cad with hormone receptor content, DNA ploidy pattern, and the expression of EGF-R, which have been reported to be prognostic factors for breast cancer.

MATERIALS AND METHODS

Patients. The surgical specimens were obtained from 120 patients with breast cancer from December 1989 to May 1991 in the Department of Surgery II, Osaka University Medical School, and the Department of Surgery, Osaka-
National Hospital. The age of the cancer patients ranged from 27 to 86 years (mean, 54.8 years). No patient had received anticancer therapy prior to the operation. Metastatic lymph nodes were also obtained from 19 patients at the time of surgery.

**Antibodies.** One hybridoma clone producing mAbs capable of inducing the disruption of cell-cell adhesion in monolayer cultures of MCF-7 cells, designated as HECD-1, was isolated as previously described (20). To detect EGF-R, anti-human EGF-R mAb (Oncor Inc, Gaithersburg, MD) was used.

**Immunohistological Staining Procedures.** The avidin-biotin-peroxidase complex method (29) was utilized for immunostaining of E-cad as described previously (26). Briefly, fresh samples including the cancerous lesions and surrounding normal glands were frozen on dry ice acetone, and then the sections (4 μm thick) were fixed with 3.6% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 30 min. Anti-human E-cad mAb, HECD-1, was applied at a dilution of 1:1000 and incubated overnight at 4°C, sequentially followed by biotinylated goat anti-mouse IgG and avidin combined in vitro with biotinylated horseradish peroxidase (Vectastain ABC Kit, Vector, Inc.). The color was developed with diaminobenzidine tetrahydrochloride supplemented with 0.02% hydrogen peroxide.

For EGF-R staining, the consecutive section was fixed with cold acetone, pretreated with normal horse serum, incubated with the antibody to EGF-R at a dilution of 1:500, and stained as described previously (30).

**Evaluation of E-cad and EGF-R Staining.** The intensity of E-cad staining for cancer cells was compared with that for normal mammary glands or ducts (Fig. 1A). Cancer cells showing E-cad staining as strong as that of normal epithelial cells were defined as E-cad positive. On the other hand, cancer cells showing much weaker or negative staining were defined as E-cad negative. The grade of E-cad expression of the tumors was semiquantitatively evaluated according to the proportion of E-cad-positive cells. When >90%, between 10 and 90%, and <10% of the cancer cells were E-cad positive, the tumors were evaluated as uniformly E-cad positive (+), heterogeneous (+/-), and uniformly E-cad negative (−), respectively (Fig. 1, B–E). The E-cad (+) tumors were classified as a type of preserved E-cad expression (Pr type), while the others (+/− and − tumors) were classified as a type of reduced E-cad expression (Rd type). The intensity of EGF-R staining of the cancer nests was compared with that for normal mammary duct and divided into the following groups: EGF-R (−), negative staining; EGF-R (+), same staining as normal duct; EGF-R (++), staining stronger than normal duct (Fig. 2).

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**Fig. 1.** Immunoreactive E-cadherin expression in human breast cancer tissues. A. normal mammary ducts. All of the epithelial cells express E-cad strongly on cell-cell boundaries. B–E, invasive ductal carcinomas. B, all of the cancerous cells strongly express E-cad. This tumor was classified as E-cad (+). C, E-cad expression differed from cell to cell. Arrow, E-cad-positive cell; arrowhead, E-cad-negative cell. This tumor was classified as E-cad (+/−). D and E, E-cad expression of all the tumor cells was much weaker or negative. These tumors were classified as E-cad (−). Arrowhead, E-cad-negative cells; arrow, cells of noncancerous epithelium. Tumors showing E-cad expression as C, D, or E are defined as Rd type. Bar 50 μm (× 100).

**Fig. 2.** Immunoreactive EGF-R expression in human breast cancer tissues. A, normal mammary ductal cells express EGF-R weakly. B–D, invasive ductal carcinomas. The cancer cells show negative (B), weak (C), and strong (D) staining for EGF-R, which were classified as EGF-R (−), EGF-R (+), and EGF-R (++), respectively. Bar 50 μm (× 100).
Immunoblot Analysis. In order to confirm our evaluation of immunohistochemical staining and molecular mass of immunoreactive E-cad, we performed immunoblot analysis on the representative samples of each staining type using the method described previously (19). Briefly, samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis using 7.5% polyacrylamide gels. After electrophoresis, proteins were incubated with mAb HECD-1, followed by incubation with 125I-labeled anti-mouse IgG (Amersham). Radiolabeled electrophoretic bands were visualized by subjecting the transfers to autoradiography. Total cellular proteins applied to each lane were adjusted to equal concentrations using the Bio-Rad protein assay kit. Human keratinocyte cells were used as a positive control.

Steroid Receptor Analysis. The contents of ER and PgR were measured by the dextran-coated charcoal method. Levels in excess of 3 fmol/mg of cytosolic protein were defined as positive for ER and PgR.

Analysis of DNA Histogram. The tissue was minced with scissors and suspended in phosphate-buffered saline containing 0.2% Triton X-100 and 0.5% RNase. The supernatant was filtered through a nylon mesh, and propidium iodide was added to the cell suspension so as to obtain a final concentration of about 50 μg/ml. More than 10⁴ cells were analyzed on a fluorescence-activated cell sorter analyzer or FACScan (Becton Dickinson Co.). The pattern on the DNA histogram was classified as diploid or aneuploid.

Histopathological Findings and Statistical Analysis. A consecutive section from each sample for E-cad staining was stained with hematoxylin and eosin for histological evaluation. The clinicopathological stage of the tumors was classified according to the TNM classification system of the International Union against Cancer (31), and their histological type was evaluated based on the World Health Organization classification (32). Their histological degree of differentiation was also graded (three categories) according to the criteria of Bloom and Richardson (33).

The χ² test was used to determine statistical significance of the results.

RESULTS

Immunohistochemical Reactivity of E-Cadherin. Expression of E-cad was clearly evident on cell-cell boundaries in normal mammary ducts and glands (Fig. 1A). Non-epithelial cells did not express E-cad molecules. In contrast to normal epithelial cells, various staining patterns were observed in cancer tissues (Figs. 1 and 4). Of the 120 primary tumors examined, 56 tumors (47%) and 64 tumors (53%) were classified as Pr type and Rd type, respectively. Those 64 Rd types consisted of 51 (+/-) and 13 (-) tumors (Table 1).

Immunoblot Analysis. Results of immunoblot analysis using mAb HECD-1 are shown in Fig. 3. Human keratinocyte cells were used as a positive control and are shown in lane NC on the immunoblot (Fig. 3). The band with highest intensity was located at 124 kDa which corresponded to that of full-size E-cad. A strong band also appeared in E-cad-positive cells that were defined as E-cad (+) using immunohistochemistry. Lanes 4-6 and 7-9 show the results of E-cad (+/-) and (-) tumors. Arrow, full size E-cad molecule at 124 kDa; the faint or totally absent on immunoblotting. Thus, the grades of E-cad expression determined was classified as diploid or aneuploid.

Correlation of E-Cadherin Immunostaining with Clinicohistological Classification. The relationship between E-cad expression and clinicohistological classification is shown in Table 1. All of the noninvasive and papillary carcinomas showed Pr-type expression, whereas 56 (58%) of 97 invasive ductal carcinomas were evaluated as Rd type, and this value was significantly higher than that in other type tumors (P < 0.01). The frequency of Rd type in grade III tumors (84%) was significantly higher than that in grade I and II tumors (38 and 51%) (P < 0.01). Thus, Rd type was observed more frequently in invasive or poorly differentiated tumors.

Concerning the tumor growth pattern, all of 34 tumors with expansive growth were evaluated as Pr type, whereas 31 (58%) of 53 tumors with infiltrative growth were evaluated as Rd type. Furthermore, all of 33 tumors with mixed growth pattern showed heterogeneous E-cad expression. Also, E-cad-positive cells formed clusters and grew expansively, but E-cad-negative cells did not form clusters and invaded by infiltration (Fig. 4). There was a strong relationship between growth pattern and E-cad expression.

The relationship between TNM staging and E-cad expression is shown in Table 2. The Rd type was observed more frequently in tumors of stages 3 and 4 than in those of stages 1 and 2 (P < 0.01). The larger tumor was the higher the frequency of Rd type. The frequency of Rd type in T3,4 tumors (71%, 22 of 31) was significantly higher than that in T1,2 tumors (47%, 42 of 89) (P < 0.05). The frequency of Rd type was high in N₁b (60%) and N₂,3 (79%) tumors, and the value in extensively metastatic tumors (N₁₈,2.7, 74%, 29 of 39) was significantly higher than that in nonmetastatic or micrometastatic (N₀,1,2.14, 43%, 35 of 81) tumors (P < 0.01).

As summarized in Table 3, we found distant metastasis in 22 patients (18 with hematogenic and 4 with metastasis to supraclavicular lymph nodes). Nineteen of them (86%) were evaluated as Rd type in 15 (+/-) tumors and 4 (-) tumors, and this value was significantly higher than that in patients without distant metastasis (P < 0.01) (Table 2).

Table 1 Correlation of E-cadherin immunostaining with histological types and tumor grades in patients with breast cancer

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Total no.</th>
<th>Pr type</th>
<th>Rd type</th>
<th>Subtotal</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive ductal carcinoma</td>
<td>97</td>
<td>41(42%)</td>
<td>47(49%)</td>
<td>94(49%)</td>
<td></td>
</tr>
<tr>
<td>with pic&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>6(5)</td>
<td>5(5)</td>
<td>11(5)</td>
<td></td>
</tr>
<tr>
<td>Lobular</td>
<td>12</td>
<td>4(25%)</td>
<td>8(50%)</td>
<td>12(25%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Papillary</td>
<td>3</td>
<td>3(100%)</td>
<td>0(0%)</td>
<td>3(100%)</td>
<td></td>
</tr>
<tr>
<td>Noninvasive</td>
<td>2</td>
<td>2(100%)</td>
<td>0(0%)</td>
<td>2(100%)</td>
<td></td>
</tr>
<tr>
<td>Others&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8</td>
<td>3(38%)</td>
<td>5(63%)</td>
<td>8(40%)</td>
<td></td>
</tr>
<tr>
<td>Tumor grade&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>40</td>
<td>25(63%)</td>
<td>15(38%)</td>
<td>40(33%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>55</td>
<td>27(49%)</td>
<td>28(51%)</td>
<td>55(46%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>16(64%)</td>
<td>9(36%)</td>
<td>25(33%)</td>
<td></td>
</tr>
<tr>
<td>Growth pattern</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expansive</td>
<td>34</td>
<td>30(94%)</td>
<td>4(12%)</td>
<td>34(100%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Infiltrative</td>
<td>53</td>
<td>42(80%)</td>
<td>11(20%)</td>
<td>53(100%)</td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>33</td>
<td>33(100%)</td>
<td>0(0%)</td>
<td>33(100%)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Significant.
<sup>b</sup> pic, predominant intraductal component.
<sup>c</sup> Others, four medullary, one mucinous, one spindle-cell type and two squamous type carcinomas.
E-CADHERIN EXPRESSION IN BREAST CANCER

We also investigated E-cad expression in metastatic lymph nodes of 19 patients and compared it with that of the primary lesions (Table 4). Eight Pr types and 11 Rd types were observed in the metastatic lesions. In 13 of the 19 metastatic lesions, E-cad was expressed in the same pattern as the corresponding primary lesions. However, in 8 patients whose primary lesions were (+/-), the metastatic lymph nodes of 3 patients were evaluated as (+) and (-) in 2 patients.

There were no correlations among E-cad expression, menopausal status, age, or location of the tumors (data not shown).

**Correlation of E-Cadherin Immunostaining with Steroid Receptors, EGF-R Staining, and DNA Ploidy Pattern.** As shown in Table 5, there was a significant correlation between EGF-R positivity and E-cad expression ($P < 0.05$). The frequency of Pr type was 40 and 71% in EGF-R (-) and (+) tumors, respectively. Thus, EGF-R positivity was more commonly observed in E-cad-positive tumors. No statistically significant difference was observed between E-cad expression and steroid receptor content or DNA ploidy pattern.

**DISCUSSION**

In the present study, we found that 64 of 120 breast cancers (53%) had impaired E-cad expression, and the majority (80%, 51 of 64) of the tumors with reduced E-cad expression showed heterogeneous expression. Reduction of E-cad expression is thought to be a suppression or impairment of transcripts of the E-cad gene or mutations in the E-cad structural gene (34). The heterogeneous E-cad expression is likely to reflect the tumor heterogeneity. However, it has been reported that highly metastatic cells have unstable E-cad expression which varies easily with cell culture conditions (35), and Mareel et al. (36) demonstrated that E-cad of Madin-Darby canine kidney cells in nude mice was reversibly down-regulated in vivo (36). Therefore, the heterogeneous E-cad expression might be due to not only the tumor heterogeneity but also unstable expression in a clone in vivo.

**Table 2 Correlation of E-cadherin immunostaining with various clinicopathological features in patients with breast cancer**

<table>
<thead>
<tr>
<th>Table no.</th>
<th>Pr type (+)</th>
<th>Pr type (+/-)</th>
<th>Pr type (-)</th>
<th>Subtotal</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>13</td>
<td>9 (69%)</td>
<td>4</td>
<td>0</td>
<td>4 (33%)</td>
</tr>
<tr>
<td>Stage 2</td>
<td>60</td>
<td>34 (57%)</td>
<td>17</td>
<td>9</td>
<td>26 (43%)</td>
</tr>
<tr>
<td>Stage 3</td>
<td>26</td>
<td>10 (38%)</td>
<td>14</td>
<td>2</td>
<td>16 (62%)</td>
</tr>
<tr>
<td>Stage 4</td>
<td>21</td>
<td>3 (14%)</td>
<td>14</td>
<td>4</td>
<td>18 (86%)</td>
</tr>
<tr>
<td>T categories$^b$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>13 (65%)</td>
<td>6</td>
<td>1</td>
<td>7 (35%)</td>
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<tr>
<td>2</td>
<td>69</td>
<td>34 (49%)</td>
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<td>35 (51%)</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
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</tr>
<tr>
<td>4</td>
<td>26</td>
<td>7 (27%)</td>
<td>14</td>
<td>5</td>
<td>19 (73%)</td>
</tr>
<tr>
<td>pN categories$^c$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>55</td>
<td>31 (56%)</td>
<td>17</td>
<td>7</td>
<td>24 (44%)</td>
</tr>
<tr>
<td>1a</td>
<td>26</td>
<td>15 (58%)</td>
<td>10</td>
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<td>11 (42%)</td>
</tr>
<tr>
<td>1b</td>
<td>10</td>
<td>4 (40%)</td>
<td>6</td>
<td>0</td>
<td>6 (60%)</td>
</tr>
<tr>
<td>2, 3</td>
<td>29</td>
<td>6 (21%)</td>
<td>18</td>
<td>5</td>
<td>23 (79%)</td>
</tr>
<tr>
<td>M categories$^d$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>98</td>
<td>52 (53%)</td>
<td>37</td>
<td>9</td>
<td>46 (47%)</td>
</tr>
<tr>
<td>1</td>
<td>22</td>
<td>3 (14%)</td>
<td>15</td>
<td>4</td>
<td>19 (86%)</td>
</tr>
</tbody>
</table>

$^a$ Significant.

$^b$ 1, ≥2 cm in greatest dimension; 2, between 2 and 5 cm; 3, ≥5 cm; 4, tumor with direct extension to skin or chest wall.

$^c$ 0, no regional lymph node metastasis; 1a, only micrometastasis (none >0.2 cm); 1b, metastasis to movable axillary nodes, any >0.2 cm; 2, metastasis to axillary lymph nodes that are fixed to one another or to other structures; 3, metastasis to ipsilateral internal mammary lymph nodes.

$^d$ 0, no distant metastasis; 1, distant metastasis (includes metastasis to supraclavicular lymph nodes).

**Fig. 4.** Immunoreactive E-cadherin expression in cancerous tissues. Arrow, E-cad-positive cell; arrowhead, E-cad-negative cell. E-cad-positive cells form clusters or epithelial structures, whereas E-cad-negative cells do not and invade by infiltration. A, arrow, mammary duct and tumor cells of intraductal component. Bar, 100 μm (X 50).

**Fig. 5.** Immunoreactive E-cadherin expression of cancer cells in metastatic lymph node. A, E-cad expression of cancer cells in lymph node metastasis which was evaluated as E-cad (+); these cells express E-cad and form nests. Bar, 50 μm (X 50). B, the lesion was regarded as E-cad (-). Arrow, tumor cells; arrowhead, lymphocytes. Bar, 50 μm (X 100).
differentiation. These observations are consistent with experiments
dedifferentiation.

In breast cancers, we also found a significant
distribution of Na⁺,K⁺-ATPase, which is an important factor for the estab
structure, and it was also reported that E-cad caused polarized distri

Significant. E-cadherin complementary DNA in fibroblastic cells generated epithelial

A significant relationship between size of tumor and E-cad expres

Further studies are necessary to explore these speculations.

cancer cells without affecting E-cad expression, should be considered
and this machinery can be affected by malignant transformation, they
The other possibility is that tumor cells express immunoreactive E-cad
expression in rat granulosa cells and regulated the cadherin

In previous reports, we (27) and Shimoyama and Hirohashi (37)
other clinical study of squamous cell carcinomas of head and neck showed that the majority (7 of 8) of infiltrated lymph nodes were E-cad negative (28). These findings are consistent with the idea that the inhibition of E-cad function enhances the possibility of release of cancer cells from the primary site. In experiments in which murine ovarian tumor cells were used, Hashimoto et al. (35) reported that E-cad expression of cells of low metastatic lines were homogeneously

However, a few Pr-type tumors had metastases, and 8 (42%) of 19 patients were found to have Pr-type lymph node metastatic lesions. We propose the following hypothesis for this incongruity of how these Pr-type tumor cells can metastasize. E-cad expression could be unstable in some tumors, as mentioned above (35, 36), and the expression might locally or temporarily diminish as in the embryo (13, 19). The other possibility is that tumor cells express immunoreactive E-cad but do not function normally, as reported previously (6, 27, 37). Since cadherin function is controlled by the cytoplasmic machinery (41, 42) but do not function normally, as reported previously (6, 27, 37). Since cadherin function is controlled by the cytoplasmic machinery (41, 42)

Table 4 Relationship between E-cadherin expression in lymph node metastasis and that in primary lesion

Table 5 Correlation of E-cadherin immunostaining with hormone receptor content, EGF-R expression, and DNA ploidy

Table 3 Properties of breast cancers with distant metastasis

Table 4 Relationship between E-cadherin expression in lymph node metastasis and that in primary lesion

In previous reports, we (27) and Shimoyama and Hirohashi (37)
showed the correlation between E-cadherin expression and histological
type of gastric carcinoma. In breast cancers, we also found a signif
icant difference in E-cad expression in relation to grade of tumor
differentiation. These observations are consistent with experiments in vitro suggesting that cadherins are important determinants of tissue
morphology. Nagafuchi et al. (38) showed that forced expression of E-cad complementary DNA in fibroblastoid cells generated epithelial
structure, and it was also reported that E-cad caused polarized distribution of Na⁺,K⁺-ATPase, which is a important factor for the establish
ment of cell polarities (39, 40). Thus, these results indicate that
loss of E-cad expression could be one of the characteristics of tumor dedifferentiation.

A significant relationship between size of tumor and E-cad expres
sion suggests two possibilities: E-cad may be lost in the course of
tumor growth or cancer cells with reduced E-cad expression may have
highly invasive characteristics. Behrens et al., using their blocking antibodies (24) and transfection technique (25), demonstrated the association between loss of cadherin function and invasive capacity in culture cells. We showed in this report that the cancer cells with preserved E-cad expression tend to grow expansively, whereas those with impaired E-cad expression tend to grow infiltratively in breast cancers. These observations suggest that the mode of cancer cell invasion may be related to E-cad expression.

Concerning the correlation between E-cad expression and metastasis, the frequency of Rd-type expression in tumors with extensive lymph node metastasis or in tumors with distant metastasis was significantly higher when compared with their counterparts. The other
cancer cells from the primary site. In experiments in which murine ovarian tumor cells were used, Hashimoto et al. (35) reported that E-cad expression of cells of low metastatic lines were homogeneously

However, a few Pr-type tumors had metastases, and 8 (42%) of 19 patients were found to have Pr-type lymph node metastatic lesions. We propose the following hypothesis for this incongruity of how these Pr-type tumor cells can metastasize. E-cad expression could be unstable in some tumors, as mentioned above (35, 36), and the expression might locally or temporarily diminish as in the embryo (13, 19). The other possibility is that tumor cells express immunoreactive E-cad but do not function normally, as reported previously (6, 27, 37). Since cadherin function is controlled by the cytoplasmic machinery (41, 42)
and this machinery can be affected by malignant transformation, they
might cause instability of cadherin-mediated cell adhesion activity
(43). Other factors, including the scatter factor, which can mobilize
cancer cells without affecting E-cad expression, should be considered
(44, 45). Further studies are necessary to explore these speculations.

Blaschuk and Farookhi (46) reported that estradiol enhanced cadherin expression in rat granulosa cells and regulated the cadherin levels in some cells. However no significant relationship was ob-

Abbreviations: i.d.c., invasive ductal carcinoma; i.pap.c., invasive papillary carcinoma, LN, lymph nodes.

Numbers in parentheses, percentages of E-cadherin-positive cells in the cancerous lesion.

In previous reports, we (27) and Shimoyama and Hirohashi (37)
showed the correlation between E-cadherin expression and histological
type of gastric carcinoma. In breast cancers, we also found a signif
icant difference in E-cad expression in relation to grade of tumor
differentiation. These observations are consistent with experiments in vitro suggesting that cadherins are important determinants of tissue
morphology. Nagafuchi et al. (38) showed that forced expression of E-cad complementary DNA in fibroblastoid cells generated epithelial
structure, and it was also reported that E-cad caused polarized distribution of Na⁺,K⁺-ATPase, which is a important factor for the establish
ment of cell polarities (39, 40). Thus, these results indicate that
loss of E-cad expression could be one of the characteristics of tumor dedifferentiation.

A significant relationship between size of tumor and E-cad expres
sion suggests two possibilities: E-cad may be lost in the course of
served between hormone receptor content and E-cad expression in breast cancer tissues. The other study in vitro suggested that EGF-R may be colocalized with cell adhesion molecules (47). In this immunohistochemical study, we also found that EGF-R tends to be positive in E-cad-positive tumors.

From these results, we conclude that E-cad may play an important role in the genesis of histological differentiation and affect the invasive or metastatic behavior of breast cancer cells in vivo. While further investigations are required to confirm the role of E-cad expression in cancer metastasis, our results suggest that E-cad expression may be used as a metastatic or prognostic marker for human breast cancer.

ACKNOWLEDGMENTS

The authors thank Associate Professor Dr. A. Okamura and Dr. N. Sakaida of the Department of Pathology, Kansai Medical University Hospital, for the histological evaluation.

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Expression of E-Cadherin Cell Adhesion Molecules in Human Breast Cancer Tissues and Its Relationship to Metastasis


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