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E-cadherin Expression Loss in T1 Invasive Ductal Carcinoma of the Breast as a Predictive Marker for Lymph Node Metastasis

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Background : E-cadherin is a transmembrane glycoprotein, which has been shown to mediate calcium-dependent epithelial cell adhesion. A loss of E-cadherin expression has been associated with the tumor invasion and metastatic potential in some human cancers. The objective of this study was to evaluate E-cadherin expression in T1 breast ductal carcinomas in order to determine whether the loss of E-cadherin expression is correlated with lymph node metastasis. **Methods :** One hundred seventy nine patients with breast invasive ductal carcinoma, measuring less than 2 cm, were enrolled in this study. The subjects were divided into two groups on the basis of the status of the ipsilateral axillary lymph node, T1N1 (lymph node positive, n=91) or T1N0 (lymph node negative, n=88). None of the patients in this study had undergone preoperative chemotherapy. Formalin-fixed paraffin-embedded tissue sections of the primary breast cancers were stained by immunohistochemistry, using a mouse monoclonal antibody against E-cadherin. E-cadherin expression was designated as either positive (complete membranous staining) or negative (absent or incomplete membranous staining). **Results :** Benign breast parenchyma adjacent to invasive carcinoma was positive for E-cadherin. The loss of E-cadherin expression in the tumor was observed in 42% of patients of the T1N1 group, and in 24% of the T1N0 group. There was a significant correlation between the loss of E-cadherin expression and lymph node metastasis in the examined breast invasive ductal carcinomas (p=0.011). **Conclusions :** Our findings suggest that E-cadherin is an important molecule with regard to both tumor cell adhesion and metastasis, and its absence may constitue an early event in metastatic development. Therefore, E-cadherin may be a useful predictive marker for nodal metastasis in patients suffering from invasive ductal carcinoma.

Key Words : E-cadherin; Invasive ductal carcinoma; Breast

Cell-cell adhesion plays a critical role in both the establishment and maintenance of normal cell polarity and in cell society. The development of malignant tumors is generally characterized by invasion and distant metastasis, both of which are considered to be later steps in carcinogenesis. Invasion and metastasis consist of a series of sequential steps involving host-tumor interactions. In order to form a metastatic nodule, the tumor cells are required to detach themselves from the primary cancer nests, invade the surrounding host tissue, and enter the circulation. The dissociation of cancer cells from the cancer nests is an essential step in this process, and is believed to be attributable to changes occurring in cell-cell adhesion.[1] Therefore, adhesion molecules are surmised to perform important functions in both cancer invasion and metastasis. Human cancers appear to exhibit both reversible and irreversible mechanisms for the inactivation of the cell-cell adhesion system.

The cadherins comprise a family of functionally related transmembrane glycoproteins, and include E-cadherin, P-cadherin and N-cadherin. The cadherins form complexes with cytoplasmic proteins, also referred to as catenins, and together with the actin filaments constitute the intercellular adherens junction. The E-cadherin gene, which is located on chromosome 16q22.1, is a primary regulator of morphogenesis.[2] E-cadherin mediates calcium-dependent homotypic cell-cell adhesion, thereby contributing to the maintenance of normal epithelial adhesion and histological structure.[3,4] The E-cadherin molecules are located within the adherens junctions as dimmers which, in the presence of calcium, can form 'zipperlike' complexes with the E-cadherin molecules in adjacent cells.[5,6] E-cadherin is believed to act as an 'invasion suppressor' in the cancer cells.[7,8] The loss of E-cadherin expression is believed to facilitate the detachment of the tumor cells from primary tumors. Several studies have reported a strong

correlation between the loss of E-cadherin and the invasiveness of tumors *in vitro*,[9] as well as with the advanced stages of a wide range of tumors, including tumors of the prostate,[10] stomach,[11] head and neck,[12] and breast.[13-15] The loss of E-cadherin has also been loosely associated with metastatic potential in cases of breast cancer.[14,15] Even small or low grade breast tumors have been determined to exhibit reduced levels of E-cadherin expression; the attenuation or loss of E-cadherin expression has been suggested to be an early event in such cases.[16] In this study, we have evaluated E-cadherin expression by immunohistochemistry in T1 breast ductal carcinoma and assessd the possibility that this is correlated with lymph node metastasis.

MATERIALS AND METHODS

Case selection

We searched the database of the M.D. Anderson Cancer Center (Texas, U.S.A.) for cases of invasive ductal carcinoma, NOS, of the breast, which measured less than 2 cm (T1, TNM system). Formalin-fixed paraffin-embedded blocks of the 179 cases were then retrieved from the surgical files. Our study group included 91 cases of ipsilateral node positive (T1N1) and 88 cases of node negative (T1N0) patients. None of the patients had undergone preoperative chemotherapy. H & E stained slides from these cases were reviewed, and one representative block of each primary lesion was selected for further immunohistochemical analysis.

Immunohistochemical stey

For our immunohistochemical analysis, we utilized the standard avidin-biotin indirect immunoperoxidase method (Santa Cruz Biotechnology Kit). Formalin-fixed paraffin-embedded blocks were cut and positioned on p-lysin labeled slides, after which the slides were baked overnight at 60℃ in an oven in order to retrieve the antigens. After deparaffinzation, endogenous peroxidase activity was blocked by 5 min of incubation in 3% H_2O_2 in methanol at room temperature. The primary antibody, a mouse monoclonal antibody against E-cadherin (Zymed Laboratories Inc, San Francisco, CA) was applied at a 1:20 dilution for 1 h at room temperature. After rinsing the slides in PBS, the biotinylated secondary IgG antibody was applied for 30 min at room temperature. The slides were then rinsed in PBS, and avidin conjugated with horseradish peroxidase (ABC reagent) was applied for 45 min at room temperature. The chromogen 3,3′ -diaminobenzidine (Research Genetics, Huntsville, AL) was then added to the slides, and the color reaction was observed under light microscopy. The reaction was discontinued by the immersion of the slides in deionized water. The slides were then counterstained with hematoxylin, and mounted.

Evaluation of Immunostaining

The sections were examined under light microscopy. The staining patterns were assessed without any prior knowledge of the lymph node status of the relevant cases. The normal breast tissues adjacent to the tumor areas were employed as internal controls. E-cadherin expression was designated as either positive (diffuse complete membranous staining) or negative (absent or incomplete membranous or cytoplasmic staining). Two pathologists (E.Kim and A.Sahin) independently assessed the results of the staining.

Statistical analysis

We utilized the chi-square test in order to determine whether any association existed between lymph node status and the loss or presence of E-cadherin. The threshold for significance was set at 5% level. In addition, we also calculated the odds ratio and a corresponding 95% confidence interval.

RESULTS

The normal breast ducts and acini adjacent to the tumor were determined to be positive for E-cadherin in all cases, in which the benign tissue was available for review. We observed no staining in the breast stroma. E-cadherin expression was localized to the cell membrane, with intense staining at the intercellular junctions (Fig. 1). All in situ areas of ductal carcinoma adjacent to the invasive tumor were E-cadherin positive (Fig. 2). E-cadherin expression in the invasive component was positive in a total of 120 cases (67%). Loss of E-cadherin expression, as evidenced by no staining or incomplete membranous or cytoplasmic staining, was observed in 38 of the cases in the T1N1 group (42%) and 21 cases in the T1N0 group (24%) (Fig. 4). Table 1 provides a summary of the results of E-cadherin expression in breast invasive ductal carcinomas. We noted a significant correlation between the loss of E-cadherin expression and lymph node metastasis in T1 breast ductal carcinomas (p=0.011).

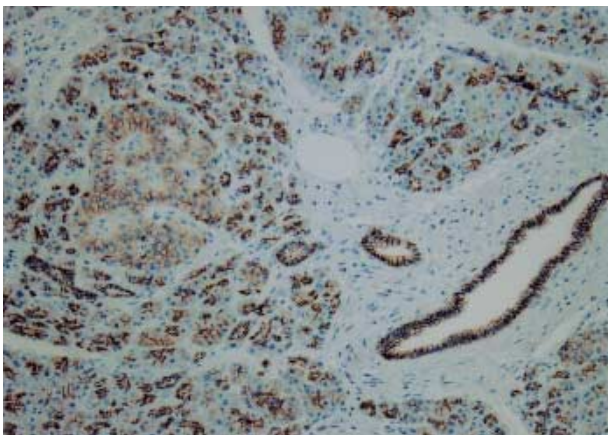

Fig. 1. E-cadherin immunostaining shows intense positivity along the cytoplasmic membrane in the normal breast (×100).

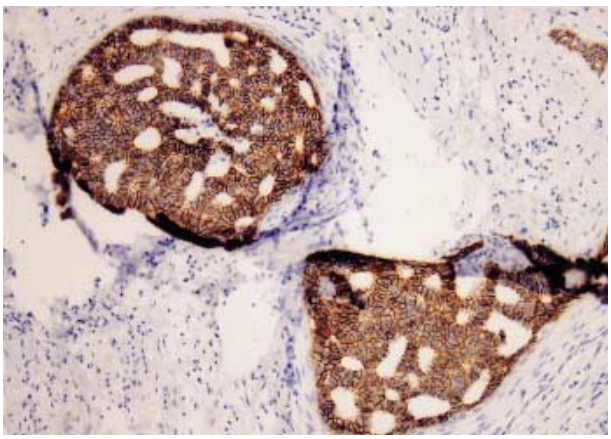

Fig. 2. Ductal carcinoma in situ shows E-cadherin positivity (×200).

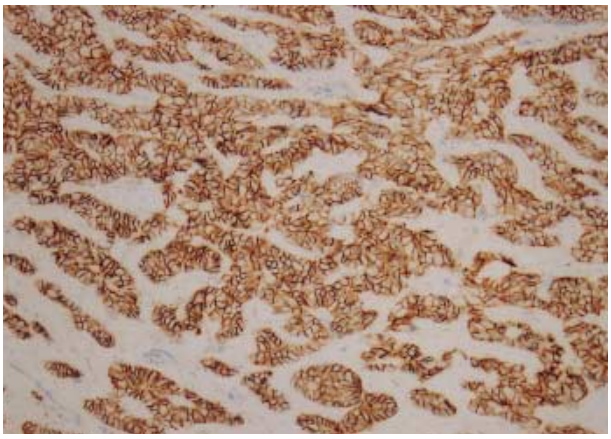

Fig. 3. Infiltrating ductal carcinoma shows E-cadherin positivity (×200).

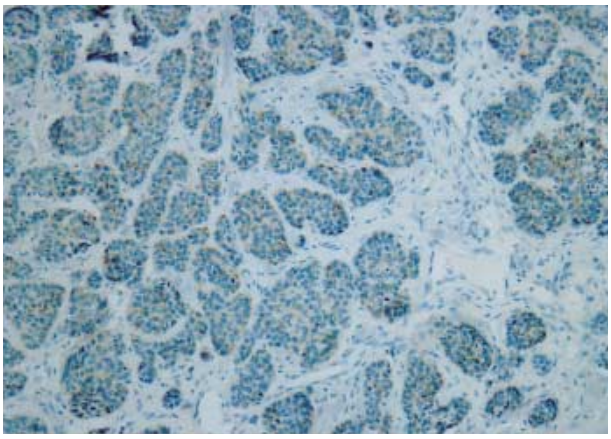

Fig. 4. E-cadherin immunostaining shows no or incomplete membranous staining in infiltrating ductal carcinoma (×100).

Table 1. Results of E-cadherin expression in T1 ductal carcinomas of the breast

	T1N1[a]	T1N0[b]
E-cadherin (-)	38 (42%)	21 (24%)
(+)	53 (58%)	67 (76%)
Total	91	88

[a]TNM system, tumor size less than 2 cm with lymph node positive; [b]TNM system, tumor size less than 2 cm with lymph node negative.

DISCUSSION

In the management of breast cancer, lymph node status appears to be the most important factor in determining therapeutic modalities. Therefore, studies have classically focused on the identification of tumors exhibiting metastatic potential in cases of breast cancer.[16] As an initial step in metastatic process, tumor cells must detach themselves from the primary site. E-cadherin has been suggested to perform an important function in the activation of the mechanism underlying the detachment of tumor cells from the primary sites. E-cadherin's potential as an invasion suppressor in epithelial tumorigenesis has been supported by several *in vitro* studies.[7-9] Also, Oka H *et al*.[11] have demonstrated a tendency toward expansile growth in human breast tumor tissues in which E-cadherin expression is retained, as opposed to the infiltrative growth associated with tumor tissues lacking E-cadherin expression.

The significance of E-cadherin expression in breast cancer remains rather controversial. Some authors have demonstrated correlations between reduced E-cadherin expression and lymph node metastasis, as well as a host of other prognostic markers and patient survival rates,[17-19] whereas others have failed to determine the existence of any such relationship.[20,21] Although the proportion of E-cadherin negative tumors in the present study could not be compared with the results of previous studies, due to the fact that different scoring systems were used in those stud-

ies, our results are still appear to be generally consonant with other data, in that we also correlated the absence of E-cadherin expression with lymph node metastasis. We interpreted the cytoplasmic staining of E-cadherin as negative, as the observed intracytoplasmic E-cadherin was considered to represent non-functioning protein, which can be located in the Golgi apparatus, rather than in the cell membrane.[22]

The E-cadherin mediated cell adhesion system in cancer cells can be inactivated by multiple mechanisms, including mutations in the E-cadherin gene, promoter hypermethylation, and dysfunctions in the normal expression of the E-cadherin molecule, often due to alterations in its associated proteins, including catenins.[23] The absence of E-cadherin due to genetic mutations is a characteristic features of lobular carcinomas.[7] The loss of E-cadherin expression in invasive ductal carcinomas appears to be fairly variable, and is usually attributed to be epigenetic mechanisms, rather than to genetic mutations.[24] Downregulated E-cadherin expression may be a transient phenomenon in the process of tumor progression. Several studies have demonstrated re-expression of E-cadherin in metastatic sites of breast cancer and prostate cancer tissues.[25-27] Although the mechanisms and biological roles of E-cadherin re-expression at metastatic sites have yet to be clearly elucidated, it seems possible that the re-expression of adhesion molecules by tumor cells after release from the primary site might be crucial to the metastasis and survival of the tumor cells in remote organs.

In this study, we elected to examine early stage breast carcinomas, which were believed to express the phenotype most likely to be important in invasion and metastasis, prior to the incidence of further unrelated genetic alterations or phenotypic changes. We determined that there were significant differences in E-cadherin expression between the T1N1 and the T1N0 groups. The loss of E-cadherin expression was strongly correlated with lymph node metastasis. The odds that an E-cadherin negative subject would also exhibit positive lymph nodes was determined to be 2.29 times that of an E-cadherin positive subject. Although the loss of E-cadherin expression is strongly associated with lymph node metastasis, as shown in this and in other studies, over half of the T1N1 group was observed to retain E-cadherin expression. This indicates that the loss of E-cadherin expression is not the sole factor involved in the metastatic process.

In summary, this study was designed to determine the significance of E-cadherin expression loss with regard to the metastatic process in breast cancer. We elected to study early stage carcinomas, in an attempt to minimize the interference posed by unrelated genetic and phenotypic events occurring during the progression of tumors. Although further study is clearly warranted in order to determine the other factors relevant to metastasis, our results suggest that the loss of E-cadherin expression is clearly one of the early event in metastatic development. This phenomenon, then, may prove useful as a predictive marker for lymph node metastasis in cases of T1 breast ductal carcinoma.

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