Expression of E-Cadherin in Human Ductal Breast Cancer Carcinoma In Situ, Invasive Carcinomas, their Lymph Node Metastases, their Distant Metastases, Carcinomas with Recurrence and in Recurrence

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Abstract. Background: Breast cancer cells can invade and generate metastasis via either lymphatic or blood vessels. E-cadherin mediates tumor cell-cell adhesion. Partial or complete loss of E-cadherin expression correlates with poor prognosis in breast cancer patients. In this study, the expression of E-cadherin was examined in mammary ductal carcinoma in situ, invasive breast carcinomas without metastasis, invasive carcinomas with their lymph node and distant metastases and invasive carcinomas with local recurrence in breast cancer tissue. Materials and Methods: Paraffin-embedded slides of carcinoma in situ (8 DCIS), invasive carcinomas without lymph node metastases (9 invasive ductal carcinomas), invasive carcinomas (7 invasive ductal carcinomas) with corresponding lymph node metastases, invasive carcinomas (8 invasive ductal carcinomas) with corresponding recurrence and invasive carcinomas (5 invasive ductal carcinomas) with corresponding distant metastases were investigated for E-cadherin expression. Tissue slides were incubated with monoclonal antibodies against E-cadherin and stained with the ABC-elite kit. Staining intensities were analyzed by using a semi-quantitative score. Results: A strong expression of E-cadherin in carcinoma in situ was demonstrated. Expression of E-cadherin was moderate in invasive carcinomas without metastases. However, very weak expression of E-cadherin in primary carcinoma with lymph node metastases was detected. E-cadherin expression was elevated in lymph node metastases compared to the primary tumor. Conclusion: Analysis of a tumor antigen involved in adhesion of breast cancer cells showed that there are significant differences of expression of E-cadherin between primary breast cancer cells and their metastases. Evaluation of this marker involved in cell adhesion could be a useful method for evaluating the metastatic risk in breast cancer patients.

Metastasis in breast cancer depends on the coordinated expression of adhesion molecules to remodel cell-cell and/or cell-matrix attachments and of proteolytic enzymes to degrade the surrounding extracellular matrix (1). Interactions between luminal epithelial cells and their environment are present during normal development and function of the mammary gland. Alterations of these interactions can induce abnormal intracellular signaling pathways that may affect development and progression of breast tumors (2). If breast cancer is detected at an early stage of the disease it seems to be restricted to the breast. But it may recur elsewhere when solely local treatment is applied. Due to breast screening programs and greater public awareness, 60% to 70% of all new cases of breast cancer appear without axillary lymph node involvement (3). On the other hand, sentinel lymph node biopsy (SLNB) is gaining acceptance as a new treatment method of axillary lymph node involvement alternative to axillary lymph node dissection. Badgwell et al. (4) showed that with a minimum follow-up of 24 months, patients with a negative SLN and no subsequent axillary treatment demonstrated a low frequency of disease recurrence. Lymph node involvement or confirmation of distant metastases in breast cancer always necessitates systemic treatment (5). Because the expression of adhesion related antigens on tumor cells is a strong indicator of the tumor’s ability to metastasize (6), many authors have tried to determine them in lymph nodes.
(7-13). However, examination of E-cadherin on carcinoma *in situ*, invasive carcinomas without metastasis and invasive tumors and its lymph node metastasis, or its recurrence tissue or distant body metastases have not yet been reported. E-cadherin, a Ca²⁺-dependent adhesion molecule, plays a major role in the maintenance of intercellular junctions in normal epithelial cells in most organs (14).

Invasion and metastasis of tumor cells is the primary cause for the fatal outcome of cancer diseases. A striking feature of metastatic cells is the considerable flexibility in their adhesive interactions with other cells or components of the extracellular matrix. Cell dissociation in these tumors is often accomplished by loss of function or expression of the epithelial cell adhesion molecule E-cadherin (6). Interestingly, the expression of E-cadherin showed a weak association with a high survival probability, while its relation to recurrence-free survival was not significant (15). In axillary lymph node-negative tumors, E-cadherin expression was not related significantly to survival or to recurrence-free survival (15).

Therefore the present study was designed to address the role of the adhesion-involved antigen E-cadherin in breast carcinoma *in situ*, invasive carcinoma without metastasis and carcinoma with its lymph node metastases or distant metastases or recurrence.

**Materials and Methods**

*Tissue samples.* A total of 57 formalin-fixed paraffin-embedded tissue blocks from patients, who underwent surgery for breast tumor at the First Department of Obstetrics and Gynaecology of the Ludwig Maximilians University of Munich were obtained. The mean age of the patients was 57±13 years with a median of 55±14 years. Tissue samples were classified according to histological classification of breast cancer *in situ* carcinomas (n=8 DCIS), invasive carcinomas without lymph node metastasis (n=9 invasive ductal), invasive carcinomas with corresponding lymph node metastases (n=7 invasive ductal carcinomas respectively), invasive carcinomas with corresponding recurrence (n=8 invasive ductal carcinomas) and invasive mammary carcinomas with corresponding distant metastases (n=5 invasive ductal carcinomas). All samples were investigated for E-cadherin expression.

*Immunohistochemistry.* Immunohistochemistry on paraffin sections (7 μm) of the tissue specimens was carried out as described elsewhere (12, 16). Briefly, sections were incubated in methanol/H₂O₂ (30 min) to inhibit endogenous peroxidase activity, washed in phosphate-buffered saline (PBS, 5 min) and treated with goat serum (20 min, 22°C) to reduce non-specific background staining. Incubation with the primary antibody (Table I) was performed overnight at 4°C. Sections were then thoroughly incubated with the biotinylated secondary anti-mouse antibody (1 h, 22°C) and avidin-biotinylated peroxidase (45 min, room temperature). Between each step, sections were washed with PBS (pH 7.4).

Peroxidase staining reaction was carried out with diaminobenzidine/H₂O₂ (1 mg/ml; 5 min) and stopped in tap water (10 min). Sections were counterstained in hematoxylin (1 min) and then cover-slipped. In controls, the primary antibody was replaced with pre-immune mouse serum with positive and negative controls being included. Villous trophoblast tissue (17, 18) served as positive controls, and lobular breast cancer tissue served as negative controls (19, 20) for E-cadherin staining. All specimens were evaluated by a pathologist with experience in immunohistochemistry. The classification of the stained cells as either micrometastases or tumor cells also required the application of histomorphological characteristics of tumor cells. Nonspecific reactions or staining of endothelial cells in the marginal sinus of lymph nodes were not considered to be tumor cells. The intensity and distribution patterns of specific immunohistochemical staining reaction were evaluated by three blinded, independent observers, including a gynecological pathologist (N.S.), using a semi-quantitative score (graded as 0=none, 1=weak, 2=moderate and 3=strong staining) without knowing the pathological evaluation, the diagnosis or the standard performed pathological hematoxylin reaction of each specimen.

*Statistical methods.* The SPSS/PC software package, version 11.0 (SPSS GmbH, München, Germany), was used for collection, processing and statistical analysis of all data. Results were evaluated using the non-parametric rank sum Mann-Whitney test for comparison and assessment of significant differences of the means (SPSS, Chicago, IL, USA). *P*≤0.05 was considered to be significant.

**Results**

*Expression of E-cadherin in breast cancer tissue.* Strong expression of E-cadherin in tissue slides of carcinoma *in situ*, with all of the cases (10/10) being positive for this antigen (Figure 1a), was found. Invasive carcinoma tissue without axillary lymph node metastases showed lower expression of E-cadherin compared to carcinoma *in situ* (Figure 1b). We identified expression of this antigen in 7/9 cases of the invasive carcinomas without axillary lymph node metastases. Lower expression of E-cadherin was identified in tumor cells with lymph node metastasis (LNM) (Figure 1c). Expression of this antigen was found in 6/7 cases investigated. The staining intensity of E-cadherin in LNM (Figure 1d) was stronger than in primary tumour and comparable to that in carcinoma *in situ*. In 6/7 of the cases investigated E-cadherin expression was found in LNM. Expression of E-cadherin was much lower in invasive carcinomas with recurrence (Figure 1e). E-cadherin was expressed in 5/8 of the cases investigated. In 6/8 of the cases with recurrence tissue positive E-cadherin staining (Figure 1f) was found. In addition, an elevated intensity of E-cadherin expression was observed in invasive carcinomas with distant body metastases (Figure 1g). We found an expression of this antigen in 3/5 of the cases investigated. The staining intensity of E-cadherin in distant body metastases (Figure 1h) was comparable to that in the primary tumor and 4/5 of the cases investigated showed an E-cadherin expression in distant body metastases.
Analysis of the staining intensity (IRS score) is summarized in Figure 2. We identified significant differences in E-cadherin staining between carcinoma in situ and invasive carcinomas without axillary lymph node metastases \( (p<0.033) \). Significant differences in E-cadherin staining between carcinoma in situ and invasive carcinoma with axillary lymph node metastases \( (p<0.001) \), in carcinoma in situ and invasive carcinomas with recurrence \( (p<0.01) \) and its recurrence tissue \( (p<0.008) \) were identified. In addition there were significant differences in E-cadherin staining in invasive carcinomas with metastases and tumor tissue of lymph node metastases \( (p<0.003) \).

**Discussion**

In this study, we analysed the expression of E-cadherin in carcinoma in situ, invasive carcinomas without metastasis, invasive carcinomas with their corresponding lymph node metastases, invasive carcinomas with recurrence and in invasive carcinomas with distant body metastasis and in its corresponding metastases in breast cancer tissue. Tumor cell dissemination and development of metastases is an active biological process involving complex interaction between cancer cells, extracellular matrix, the vascular system, the immune system and the target organ (21). Adhesion molecules play a major role in this process. The adhesion process can be divided into those which reduce adhesion of the tumor cell itself and those which increase the adhesion of floating tumor cells to the vascular endothelial tissue. E-cadherin is a glycoprotein with an extracellular domain that interacts with E-cadherin molecules on adjacent cells, thereby establishing adhesion between the epithelium. The intracellular domain is associated with a complex of proteins called catenins which anchor E-cadherin to the actin cytoskeleton (22-24). In various carcinomas, plasma membrane-associated E-cadherin protein expression is reduced or even absent. E-cadherin is a potent invasion/tumor suppressor of breast cancer. Consistent with this role in breast cancer progression, partial or complete loss of E-cadherin expression has been found to correlate with poor prognosis in breast cancer patients (25).

We found strong expression of E-cadherin in carcinoma in situ in all cases investigated and a lower expression in invasive tumor cells without metastasis. Expression of E-cadherin is further down-regulated in invasive tumor cells with LNM. In the LNM itself, we identified an up-regulated E-cadherin expression which is significantly higher than in the primary tumor. E-cadherin is further significantly reduced in carcinoma with recurrence and in the recurrence tissue. In addition, a reduced, but not significantly so, expression of E-cadherin was observed in carcinoma with distant body metastasis and in the metastasis itself compared to carcinoma in situ. Reduced E-cadherin expression reduces adhesion of the primary tumor and enhances formation of disseminated tumor emboli cells. In addition to adhesion molecules, metastasis of breast cancer is also dependent on the action of proteolytic enzymes to degrade the surrounding extracellular matrix. The tumor cell-associated urokinase-type plasminogen activator system, consisting of the serine protease uPA, its substrate plasminogen, its membrane-bound receptor uPAR, as well as its inhibitors PAI-1 and PAI-2, plays an important role in these pericellular processes (10, 26-29). Further studies are necessary for the investigation of expression and function of adhesion molecules and proteolytic enzymes to obtain more information on the mechanism of metastasis.

**Conclusion**

We performed an analysis of a tumor antigen involved in adhesion of breast cancer cells. We identified significant differences of expression of E-cadherin in primary breast cancer cells and their metastases. Evaluation of a panel of tumor markers involved in cell adhesion could be a useful method for evaluating the metastatic risk in breast cancer patients.

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**References**


Figure 1. Expression of E-cadherin in (a) ductal carcinoma in situ, (b) invasive ductal carcinoma without metastasis, (c) invasive ductal carcinoma with lymph node metastasis, (d) the corresponding lymph node metastases, (e) invasive ductal carcinoma with recurrence, (f) recurrence tissue, (g) invasive ductal carcinoma with distant body metastasis and (h) distant metastases of a ductal carcinoma (all ×10 magnification).


Figure 2. Staining intensity of E-cadherin in breast cancer subtypes determined by analysis of the immunohistochemical reaction on the different tissue slides.


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