The Loss of Intercellular Adhesion Molecules in Breast Cancer: Does it Predict a Poor Prognosis?

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Key Words

Breast cancer · Adhesive molecules · Expression · Prognosis

Summary

Background: Intercellular adhesive molecules are essential for the integrity of body tissue. A tumor's potential for invasion and metastasis depends on the structural changes of those molecules through the mutations of tumor cells. The aim of this study was to evaluate the expression of E-cadherin and β -catenin in breast carcinoma, and its effect on the outcome and survival of patients. **Material and Methods:** Immunohistochemical (IHC) staining of histological sections of different types of breast cancer for E-cadherin and β -catenin was performed in 88 breast tumors. Follow-up of the patients for a period of more than 5 years or until death was possible in all cases. **Results:** IHC staining for both E-cadherin and β -catenin was strongly positive in ductal carcinoma in situ (DCIS). Complete loss of expression was shown in 71% and 61% respectively, in infiltrating lobular carcinoma. Significant difference in loss of E-cadherin and/or β -catenin expression ($p < 0.002$) was found between grade 2 and grade 3 invasive ductal carcinoma (IDC), regardless of tumor size, status of axillary nodes, age, or ethnicity. **Conclusions:** The loss of expression of adhesive molecules is correlated with the grade of IDC tumors and the type of breast tumor, and it is not an indicator of prognosis.

Schlüsselwörter

Brustkrebs · Adhesionsmoleküle · Expression · Prognose

Zusammenfassung

Hintergrund: Interzelluläre Adhesionsmoleküle sind ausschlaggebend für die Integrität von Geweben. Das Potential eines Tumors zur Gewebeinvasion und Metastasierung hängt von strukturellen Änderungen dieser Moleküle durch Mutationen in Tumorzellen ab. Ziel dieser Studie war es, die Expression von E-Cadherin und β -Catenin in Mammakarzinomen im Hinblick auf Outcome und Überleben der Patienten zu untersuchen. **Material und Methoden:** Gewebeschnitte von 88 Mammakarzinomen verschiedener Art wurden zur Detektion von E-Cadherin und β -Catenin immunohistochemisch (IHC) gefärbt. In allen Fällen war ein Follow-up der Patienten über mehr als 5 Jahre bzw. bis zum Tode möglich. **Ergebnisse:** Bei dukalen Karzinomen in situ (DCIS) war die IHC-Färbung für sowohl E-Cadherin als auch β -Catenin stark positiv. Kompletter Verlust der Expression konnte in 71%, beziehungsweise in 61% der invasiv lobulären Karzinome gezeigt werden. Ein signifikanter Unterschied ($p < 0.002$) im Verlust der Expression von E-Cadherin und/oder β -Catenin bestand zwischen invasiv dukalen Karzinomen (IDC) zweiten und dritten Grades; ein Zusammenhang mit Tumorgroße, Status der Achsellymphknoten, Alter oder Ethnizität bestand nicht. **Schlussfolgerungen:** Verlust der Expressions von Adhesionsmolekülen korreliert mit dem Grad der IDC-Tumore sowie dem Mammakarzinomstyp, besitzt jedoch keine prognostische Aussagekraft.

Introduction

Breast cancer is the most common malignancy in women, and the second cause of cancer mortality after lung cancer [1]. Breast cancer, like other malignant tumors, is characterized by uncontrolled cell growth, detachment and migration of malignant cells to invade adjacent tissues, regional lymph nodes, and distant organs, where they become an established metastasis [2]. Understanding the mechanism of intercellular adhesiveness might help elucidate the metastatic process.

Intercellular adhesion molecules that link between homotypic cells are essential for the preservation of tissue integrity. Diverse adhesion molecules and receptors control most of the human and mammalian vital phenomena, including embryogenesis, cellular differentiation and wound healing. During the last decades, there has been an increasing interest in the structure and function of these molecules that mediate cell-to-cell and cell-to-matrix adhesion in normal tissues and in malignancy. Researchers have found that adhesion molecules play a role in the behavior of invasion and metastasis of malignant tumors [3]. The adherens junction functions to maintain the normal phenotype of these cells, and it is responsible for the strong cell-cell adhesion that promotes epithelial polarity and prevents migration of epithelial cells away from their appropriate location [4, 5]. E-cadherin (E-cad), a subclass of the cadherin family, is the transmembrane component of the adherens junction in most epithelial cells. E-cad is a calcium-regulated glycoprotein that has been studied intensively. As a transmembrane molecule, its extracellular domain is involved in cell-cell adhesion, while the intracellular domain is connected to the actin cytoskeleton through the catenins (alpha, beta, and gamma catenin) [6, 7]. Loss of expression or function of any part of the E-cad-catenin-cytoskeleton complex results in the loss of intercellular adhesion, loss of polarity and architecture; thus, detached cells become able to migrate from their main location in epithelial tissue [8, 9]. Alteration in the expression of catenins can be demonstrated despite preserved, although non-functional, E-cad expression. Decrease or loss of functional E-cad in tumor tissue is thought to be a step in the metastatic behavior of malignant epithelial tumors.

In breast cancer, an inverse relationship has been found between levels of E-cad expression and metastasis, tumor grade, and outcome. Although it has been noted that decreased expression of E-cad alone may have a limited value as a prognostic indicator [10], examining the entire repertoire of cadherins and catenins expressed by tumors may, in fact, provide useful prognostic information. Functional E-cad acts as a suppressor molecule of tumor invasiveness; thus, its expression is related to the invasive character, and it has been referred to as the 'suppressor-of-invasion gene' [11]. The gene has been cloned and is located on chromosome 16q22.1 [12]. Decreased cadherin adhesive function can result from mutations in genes encoding the catenins. For example, perturbation or mutations in β -catenin that disrupt its binding to E-cad result in a non-

adhesive phenotype [13]. The loss of E-cad expression by breast cancer cells appears to involve multiple mechanisms, including complete or partial gene depletion, methylation, and chromatin rearrangement [reviewed in 10]. E-cad expression is higher in well-differentiated invasive ductal carcinomas (IDCs), as opposed to poorly differentiated or invasive lobular tumors [14, 15]. While E-cad expression has been correlated with a high degree of tumor differentiation, it has been found to be independent of lymph node status and tumor size [16]. The aim of this study was to evaluate the correlation between the loss of E-cad and/or β -catenin and the histological type and grade of breast cancer, the metastatic status of these tumors and the 5-year survival of the patients.

Patients and Methods

Patients

Included in this study were 85 patients with breast cancer who underwent surgery in 1999–2000 at our hospital. 3 patients had bilateral and 82 patients unilateral breast carcinoma. All patients were available for follow-up for 5 years after diagnosis and surgery or until death. Patient age ranged from 28 to 88 years at the time of diagnosis of breast cancer. 20% of the patients were premenopausal, and 30% were older than 70 years. 87% were Jewish, and 13% were Arabs. In each case, tissue sections of the primary tumor were prospectively prepared and reviewed by 2 pathologists. Histology showed the diagnosis of ductal carcinoma in situ (DCIS) in 10 cases, invasive lobular carcinoma (ILC) in 14 cases, IDC in 57 cases, and miscellaneous types of breast cancer (medullary, tubular, papillary, and undifferentiated carcinoma) in 7 cases. The latter were excluded because the number of this group was too small to draw conclusions. Bilateral breast cancer was found in 3 patients: 1 with bilateral DCIS, 1 with bilateral ILC, and 1 with bilateral IDC. Estrogen (ER) and progesterone (PgR) receptor expression and HER-2/neu expression were assessed in all cases.

Immunohistochemical (IHC) Analysis

Formalin-fixed, paraffin-embedded tissue blocks were prepared from all tumors included in this study. Tissue sections 5 μ m thick were cut, deparaffinized, and treated with immunoperoxidase using the avidin-biotin complex method after high temperature antigen unmasking with the microwave technique, using EDTA buffer with the specific monoclonal antibody: the monoclonal mouse anti-human estrogen α (Dako A/S, Glostrup, Denmark), monoclonal mouse antihuman progesterone receptor, clone PgR 636 (DAKO Co., Carpinteria, CA, USA), mouse anti-HER-2(c-erbB-2) antibody, clone TAB 250, mouse anti-E-cadherin, clone 4A2C7, and monoclonal mouse anti- β -catenin, clone CAT-5H10 (Zymed Laboratories, South San Francisco, CA, USA). 2 pathologists examined the specific IHC-stained sections for ER, PgR, HER-2, E-cad, and β -catenin, respectively. The intensity of the staining of each slide section was scored on a 4-tier scale (0, +, ++, +++).

Results

The expression of E-cad and β -catenin was not affected by the age or ethnicity of the patients. ER, PgR, and HER-2/neu also seem to be unrelated to the changes in the level of E-cad or β -catenin expression in the malignant breast cells. In the 10 cases of DCIS, the IHC staining was strong (+++) in 100%

Table 1. Breast tumors included in this study and the Immunohistochemical (IHC) staining results

Tumor	Breast tumors, n	Patients, n	E-cadherin		B-catenin	
			N, %	D/L, %	N, %	D/L, %
DCIS	10	9	100.0	–	90.0	10.0
ILC	14	13	29.0	71.0	39.0	61.0
IDC						
Grade 2	27	27	63.0	37.0	63.0	37.0
Grade 3	30	29	16.7	83.3	66.7	33.3
Miscellaneous	7	7	–	–	–	–

N = normal; D/L = decreased or loss of expression, DCIS = ductal carcinoma in situ; ILC = invasive lobular carcinoma; IDC = invasive ductal carcinoma.

Table 2. Immunohistochemical (IHC) staining of 14 invasive lobular carcinomas (ILC) of 13 patients in relation to tumor size, metastatic lymph nodes, and 5-year survival

Tumor (14T/13P)	E-cadherin loss	B-catenin loss	Tumor size > 2 cm	Axillary lymph node metastases	5-year survival
ILC	71%	61%	57%	43%	86%

Table 3. Loss of E-cadherin and B-catenin in invasive ductal carcinomas (IDC)

Tumor type	Patients, n	Loss of E-cad and/or B-cat, %	p	Loss of E-cad, %	p	Loss of B-cat, %	p
Grade 2	27	59.3	0.002 ^a	37.0	<< 0.001 ^c	37.0	0.788 ^b
Grade 3	30	86.7		83.3		33.3	
Tumor size < 2 cm	32	71.9	0.771 ^b	59.4	0.944 ^b	25.0	0.064 ^a
Tumor size > 2 cm	25	76.0		64.0		48.0	
Involved axillary nodes	23	43.9	0.756 ^a	69.5	0.878 ^d	30.4	0.572 ^b
Free	32	56.1		56.3		40.6	

^a Chi-square test, 1-sided.

^b Chi-square test, 2-sided.

^c Wilcoxon rank sum test, 1-sided.

^d Wilcoxon rank sum test, 2-sided.

E-cad = E-cadherin; B-cat = β -catenin.

of cases for E-cad and in 90% for β -catenin. Complete loss of E-cad expression and β -catenin was encountered in 71%, and in 61% of ILC, respectively. 57% of ILC tumors were larger than 2 cm in diameter, and axillary lymph node metastases were found in 43% of ILC cases. 57 IDC tumors included 27 grade 2 and 30 grade 3 according to the Bloom and Richardson classification (tables 1 and 2).

The 3 dimensions of each tumor were registered in the pathology report. The largest dimension of each tumor was defined in this study as the tumor size. 32 tumors (56.1%) were less than 2 cm, and 25 (43.9%) tumors were larger than 2 cm. 55 patients had undergone complete axillary lymph node dissection in addition to either mastectomy or lumpectomy according to the surgical indications and to the preference of the patient. 1 patient with bilateral breast IDC had no lymph node analysis. Metastatic involvement of axillary nodes was encountered in 23 cases (41.8%). 10 of the 30 tumors smaller

than 2 cm (33.3%) and 13 of 25 tumors larger than 2 cm (52.0%) had already metastasized to the axillary nodes at the time of surgery. IHC staining for E-cad and β -catenin was negative in 6 IDC tumors; negative staining only to E-cad was found in 10 IDC cases; and negative staining only for β -catenin in 14 tumors. Hence, a loss of expression of E-cad and/or β -catenin was detected in 30 out of 57 tumors (52.6%). Partial loss of E-cad was shown in 19 IDC tumors (33%). Complete loss of E-cad was detected in 9 tumors smaller than 2 cm, and in 7 tumors larger than 2 cm. Complete loss of β -catenin was found in 8 tumors smaller than 2 cm, and in 12 tumors larger than 2 cm. Metastatic axillary lymph nodes were found in 6 of 26 grade 2 IDC tumors (23.1%), and in 17 of 29 grade 3 IDC tumors (58.6%).

Loss of E-cad expression was found in 11 out of 32 tumors (34.4%) with negative axillary lymph nodes, and in 5 out of 23 tumors (21.7%) with metastatic nodes. Loss of β -catenin was

Table 4. Patient survival and loss of adhesive molecules expression

Survival	Loss of E-cad, %	Loss of B-cat, %	Loss of both E-cad and B-cat, %
< 5 years	71.5	57.1	83.7
> 5 years	79.2	60.0	76.0
p (Fisher's exact test)	0.253	0.207	0.685

E-cad = E-cadherin; B-cat = β -catenin.

found in 13 of 32 tumors (40.6%) with negative axillary nodes, and in 7 of 23 tumors (30.4%) with metastatic nodes. Statistical analysis showed that the loss of E-cad is more prominent in grade 3 IDC than in grade 2 IDC (Wilcoxon rank sum test, $p < 0.001$). Although the loss of β -catenin in grade 3 IDC was more common than in grade 2 IDC, the difference was statistically non-significant (chi-square test, $p = 0.788$). Comparing the loss of E-cad and/or β -catenin in grade 3 to that in grade 2 IDC also revealed a significant difference (chi-square test, $p = 0.02$). When using both tests (chi-square test and Wilcoxon rank sum test) the loss of E-cad and/or β -catenin was found to be non-related to the size of the tumor or to the involvement of axillary lymph nodes by metastases. Decreased expression of β -catenin was noticed in association with larger tumor size (> 2 cm compared to tumors < 2 cm) but statistically, it was non-significant (chi-square test $p = 0.064$) (table 3). 10 patients died less than 5 years after breast cancer diagnosis. 1 patient with ILC and 7 patients with IDC grade 3 died due to disseminated breast cancer metastases. 2 patients with IDC grade 2 died due to second primary cancer: 1 developed pancreatic cancer, and the second developed gastric cancer. Overall 5-year survival of the patients with IDC included in this study was 84.2%.

Table 4 demonstrates the IHC of IDC tumors for patients who survived less or more than 5 years and shows that the difference in loss of intercellular adhesion molecules was statistically non-significant. This may indicate that loss or preservation of intercellular adhesion molecules per se, in breast carcinomas, has no significant effect on patients' survival.

Discussion

Intercellular adhesion molecules that preserve the integrity of epithelial tissue have been intensively studied. In adult organisms, the cadherin-catenin adhesion system maintains the differentiated state of the tissues and suppresses tumor development [11, 17–20]. Alterations in the cadherin-catenin cell-cell adhesion system are associated with loss of differentiation and tumor formation, increased invasiveness, metastasis, and unfavorable prognosis for patients with breast carcinoma [21, 22]. In our study, we have examined this hypothesis in different types, grades, and stages of breast cancer. IHC staining for

E-cad and β -catenin was performed in 88 cases of breast cancer. Strong normal expression of these 2 adhesion molecules was found in all DCIS cases ($n = 10$). This result supports the fact that DCIS behaves as a non-invasive and non-metastatic disease.

Loss of E-cad and/or β -catenin was strikingly prominent in cases of ILC (71 and 61%, respectively), comparing favorably with other published reports. In 2 cases, several foci of high-grade DCIS were found adjacent to ILC. 43% of ILC cases were associated with axillary lymph node metastases. It has been suggested that loss of E-cad is an early event in lobular breast cancer, occurring even in lobular carcinoma in situ [23]. In fact, lobular carcinomas have been associated with germ line mutations in E-cad. The striking loss of E-cad in lobular carcinoma may explain the distinctive growth pattern but is not associated with excessively high rate of metastasis. E-cad loss in the majority of lobular carcinomas results from loss of heterozygosity of the E-cad gene (CDH1) together with mutations over the coding region [23–25].

The loss of E-cad and β -catenin expression in 57 cases of IDC was significantly higher in grade 3 than in grade 2 tumors. Axillary lymph node metastases were found in 23.1% (6/26 cases) of grade 2 IDC compared to 58.6% (17/29 cases) of grade 3 tumors. This result may indicate a correlation between high-grade tumor, loss of E-cad and/or β -catenin, and the metastatic features of mammary IDC. Statistical analysis did not show a correlation between loss of E-cad and/or β -catenin and the size of the primary tumor or per se nodal metastases. Paradoxical results were also found in small tumors (> 2 cm in diameter). Irrespective of the degree of tumor differentiation and size, a decreased E-cad expression was found in 34.4% non-metastatic tumors, and in 21.7% of cases with axillary nodal metastasis.

A similar paradox has been reported. It was concluded that loss of E-cad expression is not the only, or even the most important factor involved in the metastatic process. E-cad immunopositivity shows a wide range in both metastatic and non-metastatic breast tumors; thus, it is most unlikely that measurement of E-cad expression alone will be capable of accurate prognostication in an individual case. It might be explained by E-cad immunopositivity not differentiating between functional and non-functional E-cad [26]. In a study of E-cad expression in sections of normal human mammary glands and breast carcinoma, a correlation between reduced E-cad expression and a poor degree of differentiation was found. In that studies, all IDC, including poorly differentiated cases, retained some expression of E-cad in contrast to other tumor types such as colonic and prostatic adenocarcinomas and head and neck squamous cell carcinomas, which, when poorly differentiated, were reportedly often completely E-cad-negative [27–29]. Full functional activity of E-cad is dependent on catenin binding. Certain mutations in the E-cad gene or changes in the E-cad-associated cytoplasmic proteins, the catenins, might just weaken the adhesive capability of the

molecule. Thus, reduced E-cad expression in some breast cancers could be due to a direct mutation in the E-cad gene [29]. When E-cad, β -catenin, α -catenin or plakoglobin, are all analyzed as one group, reduction of at least 1 of these markers did correlate significantly with metastases of breast carcinoma [30].

In our study, 5 of 7 patients (71.3%) with metastatic IDC grade 3 who survived less than 5 years, had a decreased E-cad and/or β -catenin expression, compared to decreased expression in 87% of similar tumors of patients who survived more than 5 years. This result shows the discrepancy between loss of adhesion molecule expression and patient survival. One study of E-cad mRNA expression in breast carcinomas showed that tumors with preserved E-cad expression tend not to disseminate whereas those with impaired E-cad expression tend to metastasize [31]. It was found, in contrast to other studies [32], that the survival of non-metastatic patients correlates with E-cad expression ($p = 0.007$). Furthermore, decreased expression was related to disease-free survival for the whole population studied ($p = 0.0065$) and the non-metastatic women ($p = 0.0059$).

There are conflicting opinions about the prognostic value of altered IHC patterns of E-cad. Some studies have found a correlation between reduced E-cad expression (particularly in node-negative patients) and their survival, while others have found it of little value in predicting clinical behavior of those patients. Thus, the usefulness of E-cad alone in this aspect is still under debate. Breast cancers with reduced or missing E-cad also can exhibit reduced catenins. One study indicated that α -catenin was reduced more frequently than E-cad in breast cancer [33]. The authors suggested that α -catenin may serve as a marker for disturbances in the adhesive function of E-cad.

In our study, we did not find a statistically significant difference between smaller and larger tumors in both grade 2 and grade 3 in E-cad and/or catenin expression (table 3). Thus, it is

apparent that the size of the IDC breast tumor is not essentially associated with loss of adhesion molecule expression. Furthermore, no statistical difference was found between decreased expression and involvement of axillary lymph nodes by metastases. This result may indicate that the cadherin-catenin-cytoskeleton complex is not the only mechanism that controls the invasiveness and metastasis of breast cancer. Cell motility factors, like scatter factor/hepatocyte growth factor and acidic fibroblast growth factor, have been found to induce invasiveness of diverse epithelial cell lines in vitro without changing the E-cad expression. Such components might also play an important role in the invasion of E-cad-positive IDCs [34, 35].

Re-expression of adhesion molecules such as E-cad, α - and β -catenins in metastatic lesions is a significant finding that demonstrates the capability of metastatic cells, after their detachment and re-growth elsewhere, to re-express their adhesion molecules [36]. Increased E-cad expression in lymph node metastases of IDC was strongly associated with improved survival, whereas expression in primary tumors was not [37]. IHC assessment of E-cad expression using tissue microarray technology in a series of 1,665 cases of invasive non-lobular breast carcinoma showed that reduction of E-cad expression was associated with reduced disease-free interval and overall survival. It was found to be an indicator of poor prognosis in larger tumors, higher histological grade, and development of distant metastases. These results suggest that reduction of E-cad expression in IDC might be an important factor in disease progression, metastases, recurrence, and poor prognosis [38].

Varied results obtained by researchers might be due to the variety of the laboratory settings and the number of cases in each series. Our series is a small one, and the results differ somewhat from those of large series. Both a larger population and longer follow up might narrow the gap between our results and those of larger series.

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