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Expression of E-Cadherin Catenin and C-erbB-2 Gene Products in Invasive Ductal-type Breast Carcinomas

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Abstract

Special attention has focused on E-cadherin and the invasiveness of breast carcinoma because E-cadherin was suggested to be the major cell adhesion molecule in the mammary gland. In the cytoplasm, E-cadherin is linked to β -catenin and α -catenin which mediate the connection of the cytoskeleton. In addition, c-erbB-2 oncoprotein causes disruption of this cell adhesion system through β -catenin phosphorylation. We investigated the expression of E-cadherin, α -catenin and c-erbB-2 gene products in 66 invasive ductal carcinomas by immunohistochemistry to examine the relation between the E-cadherin mediated cell adhesion system and histological subtypes used in Japan as well as histological grading. The series included 21 papillotubular carcinomas, 16 solid-tubular carcinomas and 29 scirrhous carcinoma. There were 33 cases of grade I, 20 cases of grade II and 13 cases of grade III. We defined P&P&N as E-cadherin positive and α -catenin positive and c-erbB-2 negative cases to evaluate the preservation of the E-cadherin mediated cell adhesion system. There were only 13 cases (19.7%) of P&P&N in total. As for the frequency of E-cadherin/ α -catenin/c-erbB-2 expression and P&P&N, no significant difference between histological subtypes was found. However, those in the grade I group tended to be higher than in the other two grade groups. Regarding the rates of α -catenin positive cases and P&P&N cases, there were significant differences between the grade I group and a combination group consisting of the grade II and grade III groups. These results suggest that the E-cadherin-mediated cell adhesion system is frequently lost in invasive ductal-type breast cancers by random loss of E-cadherin/catenins or c-erbB-2 overexpression, and that the preservation of this system correlates with well differentiated morphological features.

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Key words: breast carcinoma, E-cadherin, catenin, c-erbB-2, immunohistochemistry

Introduction

A number of membranous organelles such as

desmosomes and adherens junctions guarantee the integrity of the mammary gland. They contain cell adhesion molecules to serve as adhesive elements interconnecting epithelia and making contact between

epithelial and myoepithelial cells. Among the various cell adhesion molecules, cadherins are the major force behind cell-cell adhesion. Several types of cadherins have been identified as integral membrane glycoproteins with a single transmembrane domain. Since most normal epithelial tissues in adult organisms express E-cadherin, it is suggested to be the major cadherin in epithelial cells. The extracellular domain of E-cadherin forms a Ca^{2+} -dependent contact with E-cadherin of neighboring cells in a mainly homophilic manner. The short cytoplasmic domain is linked to β -catenin, to which α -catenin binds and mediates the connection to actin fibers of the cytoskeleton¹². The physiological function of E-cadherin depends on the integrity of the entire cadherin/catenin/actin network because E-cadherin and catenins are required for interaction between the cellular homophilic contact zone and the actin cytoskeleton. In addition, phosphorylation of β -catenin causes disruption of the cadherin-mediated cell adhesion system in cancer cells, and c-erbB-2 gene product, a member of type 1 family of receptor tyrosine kinases directly associates with β -catenin phosphorylation³⁴. Therefore, catenins and the c-erbB-2 gene product as well as E-cadherin are important to investigate inactivation of the E-cadherin-mediated cell adhesion system known as an invasion suppressor system in cancer cells.

The age-adjusted death rate of breast carcinoma in Japan has been continuously rising for several decades, to be ranked as the third leading position in Japanese females in 1995⁵. Special attention has focused on the E-cadherin mediated cell adhesion system and its association with clinicopathological features in breast cancer including Japanese cases⁶⁻¹³. The results and conclusions of previous studies are occasionally inconsistent with each other, partly because catenin expression was taken into consideration as well as E-cadherin in some studies but not in others. Therefore, this study focused on the co-expression/loss of E-cadherin, α -catenin and c-erbB-2 gene products in a series of invasive ductal cancers of the breast.

According to histological typing of breast tumors by WHO¹⁴, invasive ductal carcinomas is a single histological category of invasive carcinomas, while the Japanese Breast Cancer Society classifies invasive ductal carcinoma into three subtypes, namely,

papillotubular carcinoma, solid-tubular carcinoma and scirrhous carcinoma in general rules for clinical and pathological recording of breast cancer¹⁵. In the present study, we investigated the distribution of E-cadherin, α -catenin and c-erbB-2 gene products in 66 invasive ductal carcinomas by immunohistochemistry to examine the relation between the E-cadherin/catenin cell adhesion system and histological subtypes of invasive ductal carcinoma as well as the histological grading or other clinicopathological features.

Materials and Methods

Patients and Histology

Paraffin-embedded surgical material from 66 breast cancer patients with invasive ductal carcinomas received between 1986~1995 at the Tama Nagayama hospital of Nippon Medical School were analyzed. The mean age of the patients was 51 years with 12.4 years of standard deviation. At the time of diagnosis, patients had no diagnosable distant metastases. Histopathological findings were recorded at the time of diagnosis. Tumor size and lymph node involvement were defined according to the TNM classification. Tumor diameters ranged from 4.0 cm to 0.8 cm excluding a 12 cm of exception. The mean diameter was 2.4 cm with 1.6 cm of standard deviation.

The tissue specimens were fixed in 20% buffered formalin, embedded in paraffin, sectioned at 3 μm and stained with hematoxylin and eosin. Histological type and sub-classification were determined according to the Japanese Breast Cancer Society¹⁵. The sub-classification of invasive ductal carcinoma is based on the predominant one when there are two different histological subtypes. Histological grade was obtained according to the method of Elston and Ellis¹⁶, using the scale assigned to three features: tubule formation (1 to 3), nuclear pleomorphism (1 to 3), and mitotic count (1 to 3). A numerical scoring system was used and the overall grade was derived from a summation of individual scores for the three variables.

Immunohistochemistry

The following antibodies were used: (a) HECD-1 (Takara, Tokyo, Japan) for detection of E-cadherin; (b) anti- α -catenin antibody (Sigma, St. Louis, USA)

for detection of α -catenin; (c) anti-c-erbB-2 gene products polyclonal anti-body (Nichirei, Tokyo, Japan) for detection of the c-erbB-2 oncoprotein.

For immunohistochemistry, sections were mounted on 3-aminopropylmethoxysilane (SILANE, Sigma)-coated slides, deparaffinized and rehydrated. Sections were incubated in 0.1% hydrogen peroxide to block endogenous peroxidase activity. The sections were then pretreated by heating in a microwave oven 3 times for 4 minutes for E-cadherin detection. For α -catenin detection, the sections were pretreated with 0.1% trypsin for 15 min at 37°C. To reduce nonspecific antibody binding, sections were preincubated with bovine serum albumin for 20 minutes. Thereafter, the peroxidase technique was applied. Sections were incubated overnight at 4°C with the primary antibodies (dilution for HECD-1, 1:100; for anti- α -catenin antibody, 1:1,000; for anti-c-erbB-2 antibody, 1:50). After washing with PBS, sections were incubated with biotinylated secondary antibodies for 60 minutes at room temperature. The sections were then rinsed in PBS and covered with streptavidin-horseradish peroxidase for 45 minutes. The slices were thoroughly washed and then reacted with 0.02% diaminobenzidine (DAB) (Sigma) containing 0.005% hydrogen peroxide for 2 to 7 minutes and counterstained with Meier's hematoxylin.

Large and small ducts of normal mammary tissue entrapped in the specimens were used as internal controls for expression of E-cadherin and α -catenin. Incubation with an irrelevant primary antibody served as a negative control.

Immunohistochemical scoring

Expression of the antigens was examined on the largest section of each tumor. Staining was recorded as positive when 30 or more percent tumor cells showed immunoreactivity, and as negative when less than 30% tumor cells showed immunoreactivity. In the case of c-erbB-2 gene products, only membranous reactivity was recorded as positive. Statistical analyses was performed by the $m \times n$ chi-square method.

Results

Histological grades and correlation with histological types

The series included 21 papillotubular carcinomas

(Pap), 16 solid-tubular carcinomas (Sol) and 29 scirrhous carcinoma (Sci). Representative examples of the three histological subtypes of invasive ductal carcinomas are presented in **Fig. 1**. There were 33 cases of grade I, 20 cases of grade II and 13 cases of grade III. **Table 1** shows the correlation between histological grades and types in the 66 cancers. No significant correlation between histological grades and types was found.

Immunohistochemistry of E-cadherin and α -catenin in normal breast tissue

Lobular and ductal epithelium of normal breast tissue expressed E-cadherin and α -catenin in a regular array on lateral cell borders and with diffuse distribution in the cytoplasm, respectively (data not shown).

Immunohistochemistry of E-cadherin and α -catenin in breast carcinomas

In ductal carcinoma cells, the sub-cellular distributions of E-cadherin and α -catenin were similar to normal breast tissue (**Fig. 2**). The distributions of positive cells for E-cadherin and α -catenin in cancer tissues were markedly various in each case although they tended to be frequently expressed in well-differentiated portions.

c-erbB-2 overexpression in breast carcinomas

Immunostaining of c-erbB-2 oncoprotein was positive in 24 cases (36.4%) (**Fig. 2**).

Relation of E-cadherin / α -catenin / c-erbB-2 expression with histological subtypes

E-cadherin positive cases were 47.6%, 62.5% and 55.2% in the Pap, Sol and Sci groups, respectively. α -catenin positive cases were 81.0%, 75.0% and 65.5% in the Pap, Sol and Sci groups, respectively. c-erbB-2 positive cases were 33.3%, 25.0% and 44.8% in the Pap, Sol and Sci groups, respectively. As for the frequency of E-cadherin/ α -catenin/c-erbB-2 expression, no significant difference between histological subtypes was found. We defined P&P&N as E-cadherin positive and α -catenin positive and c-erbB-2 negative cases. P&P&N were 19.0%, 37.5% and 10.3% in the Pap, Sol and Sci groups, respectively. There was also no significant association between the percentage of P&P&N and the histological subtypes (**Table 2**).

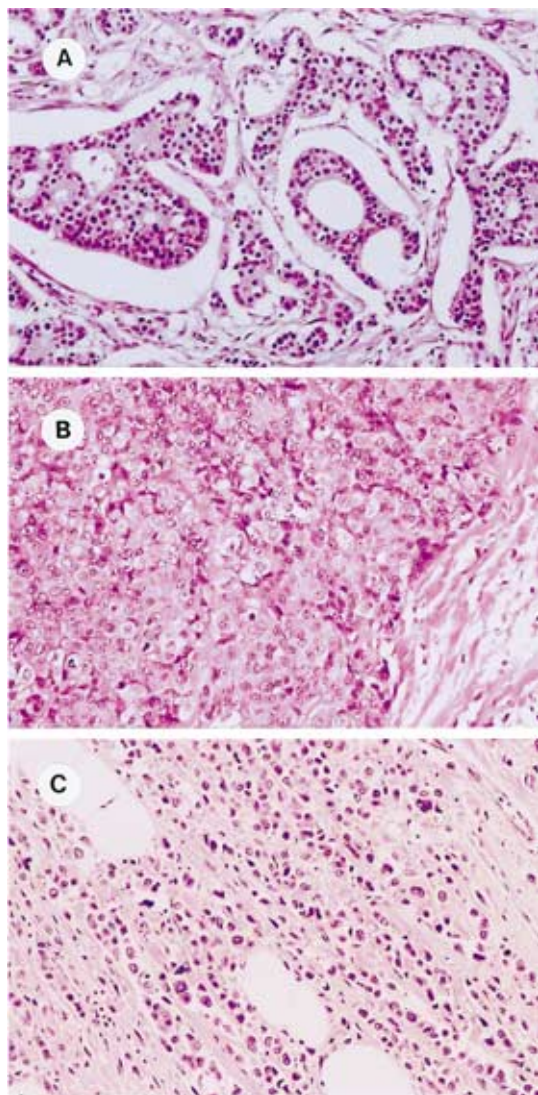


Fig. 1 Representative photographs of histological subtypes of invasive ductal-type breast carcinomas as recognized by the Japan Breast Cancer Society. A: Papillotubular carcinoma (Pap). Note the cribriform pattern. B: Solid-tubular carcinoma (Sol). Note the solid mass including scattered micro-tubular structures. C: Scirrhous carcinoma (Sci). Note the small strands of cancer cells or individual cancer cells in the fibrous stroma. Original magnification: $\times 100$ (A, B, C).

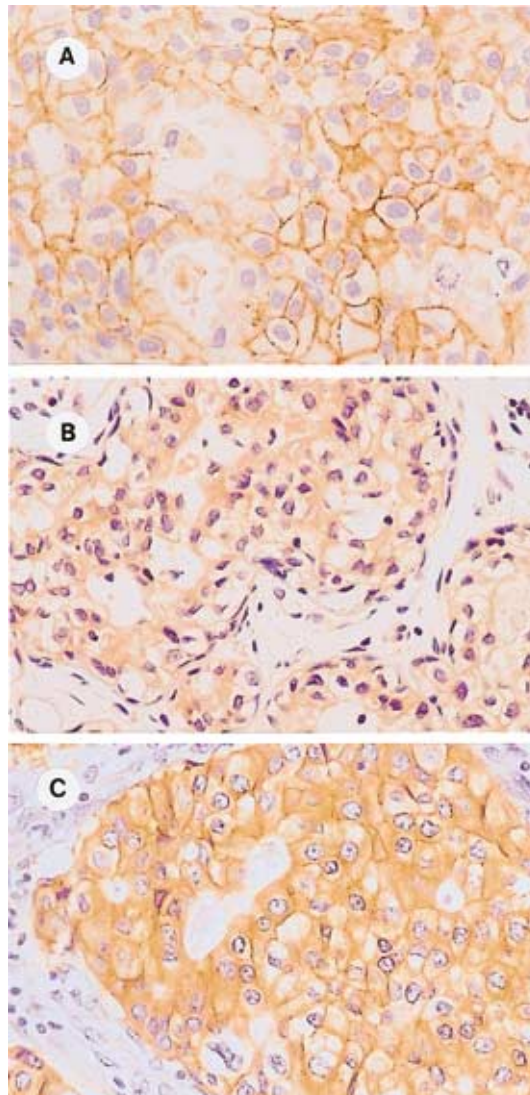


Fig. 2 Representative carcinoma cases with positive immunostaining for E-cadherin (A), α -catenin (B) and c-erbB-2 gene products (C). A: Strong membrane-bound immunoreactivity of E-cadherin. B: Diffuse immunoreactivity of α -catenin in cytoplasm. C: Strong membrane-bound and cytoplasmic immunoreactivity of c-erbB-2 gene products. Original magnification: $\times 200$ (A, B, C).

Table 1 Correlation between histological types and histological grades

	Pap	Sol	Sci	total
Grade I	12	6	15	33
Grade II	6	4	10	20
Grade III	3	6	4	13
total	21	16	29	66

Relation of E-cadherin/ α -catenin/c-erbB-2 expression with histological grading

E-cadherin positive cases were 63.6%, 40.0% and 53.8% in the grade I, grade II and grade III groups, respectively. α -catenin positive cases were 84.8%, 60.0% and 61.5% in the grade I, grade II and grade III groups, respectively. c-erbB-2 positive cases were 33.3%, 35.0% and 46.1% in the grade I, grade II and grade III groups, respectively. P&P&N

were 30.3%, 5.0% and 15.4% in the grade I, grade II and grade III groups, respectively. As for the expression rates of the three molecules, although there was no significant difference between histological grades, those of E-cadherin/ α -catenin and the P&P&N rate in the grade I group tended to be higher than in the other two groups (Table 3). Thus, a combination group consisting of the grade II and grade III groups was compared with the grade I group. Regarding the rates of α -catenin positive cases and P&P&N cases, significant differences between the grade I group and the combination group were found (Table 4).

Relation of c-erbB-2 expression with E-cadherin/ α -catenin expression

c-erbB-2 positive cases more frequently expressed E-cadherin and α -catenin than c-erbB-2 negative cases. Therefore, c-erbB-2 expression directly correlated with α -catenin expression ($p < 0.05$) and showed a similar tendency to E-cadherin expression ($p = 0.07$) (Table 5).

Relation of E-cadherin/ α -catenin/c-erbB-2 expression with lymph node metastasis

Statistical analysis did not reveal a significant correlation between E-cadherin/ α -catenin/c-erbB-2 expression and lymph node metastasis. The frequency of lymph node metastasis in the P&P&N group and the non-P&P&N group was each 23.1% (3/13) and 45.1%

(24/53). However, this difference was not significant.

Discussion

The three subtypes of invasive ductal carcinoma adopted by the Japanese breast cancer society have been widely used throughout Japan¹⁷. Papillotubular carcinoma (Pap) is characterized by the projection of papillae into spaces and includes cribriform patterns and comedo patterns. Solid tubular-carcinoma (Sol) is a solid tumor mass consisting of micro-tubular or trabecular structures and reveals expansive growth compressing the surrounding tissue and forming a sharp border. Scirrhus carcinoma (Sci) is characterized by small cancer nests or separate cancer cells accompanied by marked fibrosis. The classification is based on the predominant one when there are two different histological subtypes. Pap showed the best prognosis and Sci showed the worst one in some studies^{18,19}.

On the other hand, histological grading¹⁶, as used in the present study, has modified the original method described by Bloom & Richardson²⁰. This revised method involves semi-quantitative evaluation of three morphological features; the percentage of tubule formation, the degree of nuclear pleomorphism and an accurate mitotic count using a defined field area. A numerical scoring system is used and the overall grade is derived from a summation of individual

Table 2 Relation of E-cadherin/ α -catenin/c-erbB 2 expression with histological types

	Pap	Sol	Sci
E-cadherin	47.6% (10/21)	62.5% (10/16)	55.2% (16/29)
α -catenin	81.0% (17/21)	75.0% (12/16)	65.5% (19/29)
c-erbB2	33.3% (7/21)	25.0% (4/16)	44.8% (13/29)
P & P & N	19.0% (4/21)	37.5% (6/16)	10.3% (3/29)

P & P & N: E-cadherin positive, α -catenin positive and c-erbB2 negative cases

Table 3 Relation of E-cadherin/ α -catenin/c-erbB 2 expression with histological grading

	Grade I	Grade II	Grade III
E-cadherin	63.6% (21/33)	40.0% (8/20)	53.8% (7/13)
α -catenin	84.8% (28/33)	60.0% (12/20)	61.5% (8/13)
c-erbB2	33.3% (11/33)	35.0% (7/20)	46.1% (6/13)
P & P & N	30.3% (10/33)	5.0% (1/20)	15.4% (2/13)

P & P & N: E-cadherin positive, α -catenin positive and c-erbB2 negative cases

scores for the three variables. Patients with grade I tumors showed a significantly better survival than those with grade II and III tumors¹⁶. We expected that E-Cadherin and/or α -catenin would have been most often lost in Sci or grade III cases in the three histological subtypes or grades, and that c-erbB-2 gene products would have been most frequently expressed in these two groups. While no particular tendency or no significant correlation was found between the expression rates of E-cadherin/ α -catenin/c-erbB-2 and the histological subtypes (**Table 2**), those of E-cadherin, and α -catenin in the grade I group tended to be higher than in the grade II or III groups. In addition, there was an inverse tendency as for c-erbB-2 expression (**Tables 3, 4**). The reason why the results based on histological grades differed from those based on histological subtypes is unclear. However, as for the present series, histological grades did not significantly correlate with histological subtypes (**Table 1**). Usually, "histological classification" of invasive breast cancer means invasive ductal carcinoma and invasive lobular carcinoma, except in Japan⁷²¹. To date, no report has mentioned the histological subtypes in invasive ductal carcinoma adopted by the Japanese Breast Cancer Society in studies of E-cadherin so far as is known. The present study might be the first to

report on the histological subtypes used in Japan and the E-cadherin mediated cell adhesion system in invasive ductal carcinomas of the breast.

The direct correlation between c-erbB-2 expression and α -catenin/E-cadherin expression (**Table 5**) is not consistent with one study's findings that c-erbB-2 overexpression down-regulated E-cadherin expression in a non-tumorigenic human mammary epithelial cell line²². In previous studies, c-erbB-2 and E-cadherin expression were reported to be not significantly related, and to be independent of each other in invasive ductal carcinomas^{21,23}.

As described in the introduction section, the physiological function of E-cadherin depends on the integrity of the entire cadherin/catenin/actin network. In addition, the c-erbB-2 gene product, a member of receptor tyrosine kinases, could disrupt this cell-adhesion system through β -catenin phosphorylation. Therefore, in the present study, being positive for E-cadherin and α -catenin, and simultaneously negative for the c-erbB-2 gene product in a single case is at least essential for preservation of the E-cadherin-mediated cell adhesion system. Therefore, we defined P&P&N (**Tables 3, 4**). Although it accounted for only 13 cases (19.7%) in total of the present series, the rate of P&P&N was significantly higher in the Grade I group than in the combination group consisting of the Grade II and Grade III groups (**Table 4**). The P&P&N group showed markedly less lymph node metastasis than the non-P&P&N group, but there was no significant difference. There is a report, done from the point of view we share, that axillary lymph node metastases were completely lacking only in the group (n=6) where expression of E-cadherin, and α - and β -catenin were preserved in 74 cases of an invasive ductal carcinoma series²¹.

The results in the present study suggest that the E-cadherin-mediated cell adhesion system is

Table 4 Relation of E-cadherin/ α -catenin/c-erbB 2 expression with combined histological grading

	Grade I	Grade II or III
E-cadherin	63.6% (21/33)	45.4% (15/33)
α -catenin	84.8% (28/33) *	60.6% (20/33) *
c-erbB2	33.3% (11/33)	39.3% (13/33)
P & P & N	30.3% (10/33) **	9.2% (3/33) **

P & P & N: E-cadherin positive, α -catenin positive and c-erbB2 negative cases *, ** p < 0.05

Table 5 Relation of c-erbB 2 expression with E-cadherin/ α -catenin expression

	E-cadherin positive		α -catenin positive
c-erbB2 positive (no.)	24	70.8%	91.6% *
c-erbB2 negative (no.)	42	45.2%	61.9% *

* p < 0.05

frequently lost in invasive ductal-type breast cancers by random loss of E-cadherin/catenins or c-erbB-2 overexpression, and that the preservation of this system correlates with well differentiated morphological features. Some shortcomings of the present study are as follows. Firstly, the number of cancer cases was small; secondly, β -catenin was not examined; thirdly, findings about long-term prognoses are unclear. Further investigations are required to work on these problems.

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