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# Frequent Loss of α Catenin Expression in Scirrhous Carcinomas with Scattered Cell Growth

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**To investigate the mechanisms of disruption of cell-cell contact in scirrhous carcinoma cells, the expression of both E-cadherin and α catenin, which is an intracellular cadherin-binding molecule, were determined in scirrhous-type adenocarcinomas of the stomach and breast using immunohistochemical and immunoblotting techniques. The losses of E-cadherin expression in gastric and breast scirrhous adenocarcinomas were 18.1% and 0%, respectively, and those of α catenin expression were 54.6% and 75%, respectively. Frequent loss of α catenin expression occurred in scirrhous carcinomas with scattered cell growth in the stomach and the breast and showed no organ specificity. In addition, all the infiltrating lobular carcinomas, which also infiltrate the stroma as single cells, showed no E-cadherin or α catenin expression. These findings suggest that down-regulation of either α catenin or E-cadherin plays a critical role in the disruption of cell adhesion in carcinomas with scattered cell growth.**

Key words: Cell adhesion molecule — α catenin — E-cadherin — Scirrhous gastric adenocarcinoma — Infiltrating lobular carcinoma

Cell-cell adhesion molecules are believed to play important roles in organogenesis, signal transduction and ontogenesis from the early developmental stages, and various different types of cell adhesion molecules have been identified.[1–5] Among them, one called E-cadherin, which is located at the *zonula adherens,* is functionally important for epithelial architecture maintenance.[6] The cytoplasmic domains of different cadherin family members are now known to interact with distinct groups of cytoplasmic proteins, namely α, β and γ catenins.[7–13] Cadherins anchor and organize the actin cytoskeleton via catenin molecules which are necessary for cadherin functions. Recently, we have discovered that a cancer cell line, PC 9, with insufficient cadherin functions expressed E-cadherin, β catenin and γ catenin but not α catenin.[14] Transformation of αN-catenin, which is a subtype of α catenin, into PC 9 cells resulted in acquisition of tight cell-to-cell adhesion and organoid structure.[15] These data suggested that α catenin is a key regulator of cadherin function and multicellular organization.

Scirrhous carcinoma, which is defined as a cancer possessing more or less productive stromal fibrosis, is observed frequently in the stomach and breast.[16–19] Scirrhous carcinoma of the stomach and breast has characteristic histological features, in that most of the cancer cells show marked scattered cell growth with loose cell-to-cell connections and infiltrate surrounding fibrous tissue as single cells without a glandular structure. Our previous study on the expression of cell adhesion molecules revealed that the complete form of E-cadherin, was expressed in many scattered-type scirrhous adenocarcinoma cells.[20,21] This result suggested that other molecules involved in the cadherin-associated cell adhesion apparatus may be altered in such scirrhous carcinomas. There is another type of breast carcinoma, lobular carcinoma, which appears to originate from the mammary gland acinar cells,[18,19] that also has loose cell-to-cell connections and infiltrates the stroma in strands. In an attempt to determine the possible mechanism of cell-to-cell connection disruption in carcinoma cells, we investigated E-cadherin and α catenin expression in adenocarcinomas of the stomach and the breast, including scirrhous carcinomas showing scattered cell growth.

## MATERIALS AND METHODS

**Materials and histological classification** Fresh cancer tissues as well as individual noncancerous tissues were obtained at the time of surgery in the National Cancer Center Hospital from 94 primary gastric carcinomas and 34 primary breast carcinomas. They were then fixed with Amex and embedded in paraffin as described previously.[20,21] Three μm serial sections were cut and used for immunohistochemical staining. Gastric cancers were classified according to the general rules of the Japanese Gastric Cancer Study.[22] They were subclassified into two

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types of adenocarcinoma according to their histological types: differentiated adenocarcinoma, which comprises papillary adenocarcinoma and well and moderately differentiated tubular adenocarcinoma, and undifferentiated adenocarcinoma, which comprises poorly differentiated adenocarcinoma, mucinous adenocarcinoma and signet-ring cell carcinoma. Undifferentiated carcinomas of the stomach and infiltrating ductal carcinomas were classified into solid and scattered types according to the histological growth pattern. The undifferentiated type of gastric adenocarcinomas with scattered cell growth corresponds well to scirrhous carcinoma of the stomach.

**Immunohistochemistry and immunoblotting analysis** The monoclonal antibody, anti-human E-cadherin mouse monoclonal antibody, HECD-1, was produced and characterized previously. The specificity of anti-$\alpha$ catenin rat monoclonal antibody, $\alpha$-18, was examined by using Western blotting and immunohistochemical techniques. The techniques of immunostaining with the monoclonal antibodies, HECD-1 and $\alpha$-18, used in this study, are described elsewhere.[20, 21] For immunohistochemistry, 3 $\mu$m serial sections were deparaffinized and rinsed with phosphate-buffered saline containing 2 m$M$ $CaCl_2$ (PBS(+)). The sections were washed with PBS(+), then treated with 2% swine serum in PBS(+) for 30 min, followed by incubation with HECD-1 or $\alpha$-18 for 2 h. They were then incubated with peroxidase-conjugated anti-mouse or rat antibody. For the immunoblotting analysis, fresh, normal gastric mucosa obtained at surgery was extracted using lysis buffers containing 1% (v/v) Nonidet P-40, 1% (v/v) Triton X-100, 1 m$M$ phenylmethanesulfonyl fluoride (PMSF) and 5 m$M$ $CaCl_2$ in phosphate buffer. Equal amounts of tissue proteins were electrophoresed in 7.5% sodium dodecyl sulfate (SDS)-polyacrylamide gel and the proteins were transferred onto nitrocellulose membranes, which were incubated with the HECD-1 and $\alpha$-18 antibodies. The immunoreactions were visualized using ECL (Amersham), according to the method recommended by the supplier.

## RESULTS

**Specificity of the monoclonal antibodies** The results of immunoblotting analysis and immunohistochemical staining using HECD-1 and $\alpha$-18 antibodies are shown in Fig. 1 for human normal gastric mucosa. The HECD-1 and $\alpha$-18 antibodies recognized single bands of 124 kD and 102 kD molecules, respectively (Fig. 1A). The $\alpha$-18 antibody did not react with vinculin, which is a 130 kD $\alpha$ catenin homologous with intracytoplasmic protein. The

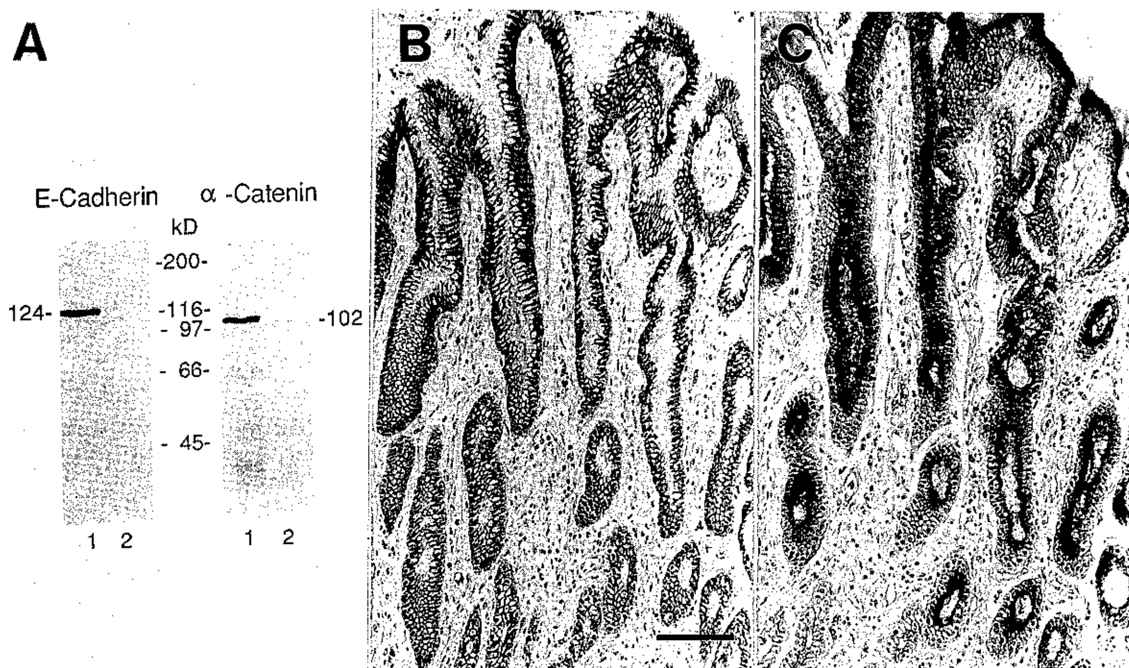


Fig. 1. Immunoblotting and immunohistochemical detection of E-cadherin and $\alpha$ catenin in normal gastric mucosa. In immunoblot detection (A), lanes 1 were stained with HECD-1 for E-cadherin and $\alpha$-18 for $\alpha$ catenin and lanes 2 with non-immunized normal mouse and rat immunoglobulin, respectively. Immunohistochemical staining of E-cadherin (B) and $\alpha$ catenin (C) in normal gastric mucosa. The sections were counterstained with hematoxylin. Scale bar, 100 $\mu$m.

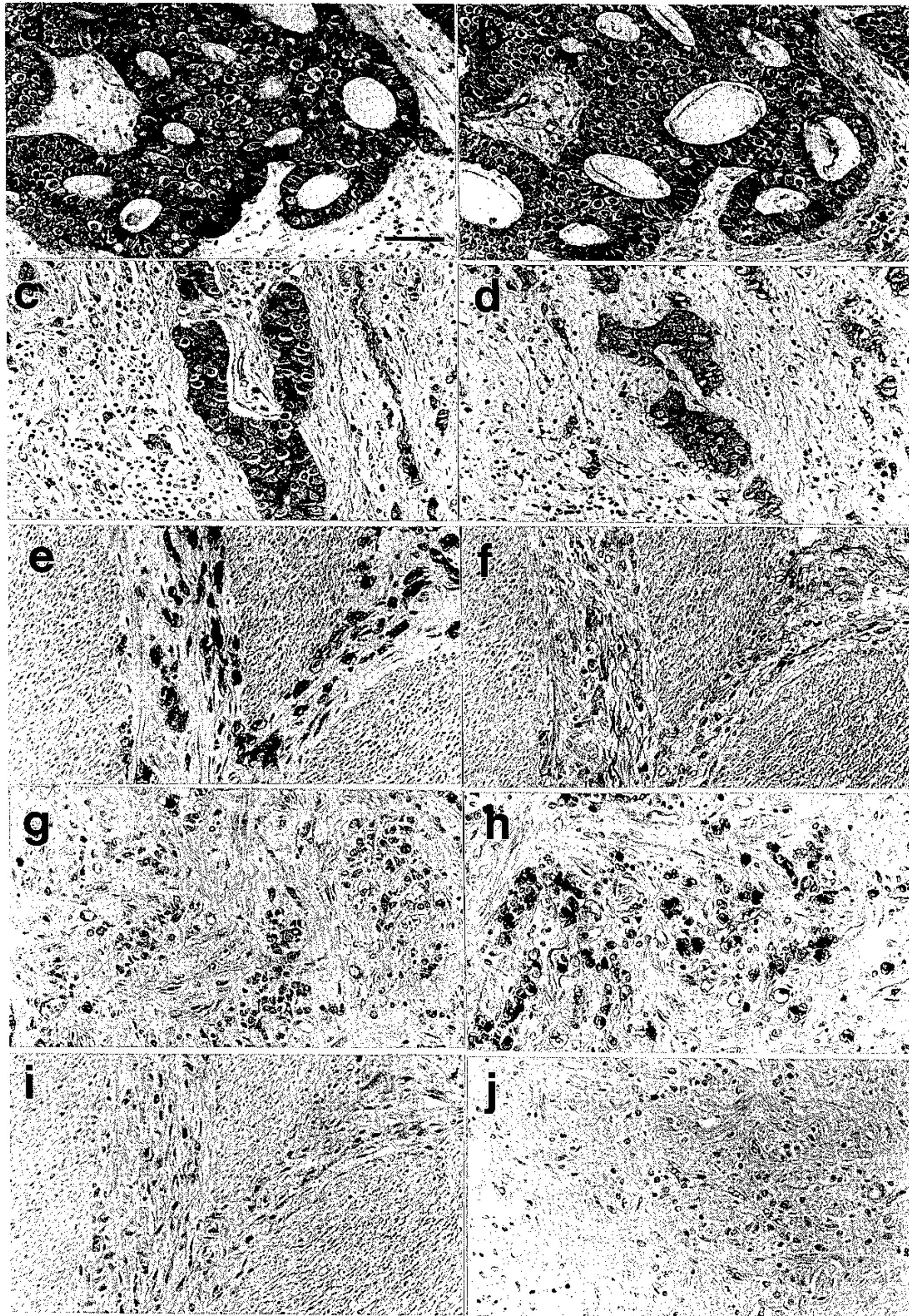


Fig. 2. Immunohistochemical staining of E-cadherin and $\alpha$ catenin in various gastric cancers. Differentiated adenocarcinoma (a, b), solid-type undifferentiated adenocarcinoma (c, d), and scattered-type undifferentiated adenocarcinoma (e. f, g, h, i, j), were stained with HECD-1 for E-cadherin (a, c, e, g) and $\alpha$-18 for $\alpha$ catenin (b, d, f, h) antibodies. Normal mouse (i) and rat immunoglobulin (j) were used for negative control staining. The sections were counterstained with hematoxylin. Scale bar, 20 $\mu$m.

Table I. Expression of $\alpha$ Catenin and E-Cadherin in Gastric Carcinoma

| $\alpha$ catenin expression | | ++ | ++ | ++ | + | + | + | − | − | − |
|---|---|---|---|---|---|---|---|---|---|---|
| E-cadherin expression | | ++ | + | − | ++ | + | − | ++ | + | − |
| Differentiated-type adenocarcinoma | 33 | 21 | 0 | 0 | 3 | 9 | 0 | 0 | 0 | 0 |
| Undifferentiated-type adenocarcinoma | | | | | | | | | | |
| Solid type | 17 | 8 | 0 | 0 | 3 | 1 | 0 | 0 | 5 | 0 |
| Scattered type | 44 | 7 | 0 | 3 | 7 | 2 | 1 | 12 | 7 | 5 |
| Total | 94 | 36 | 0 | 3 | 13 | 12 | 1 | 12 | 12 | 5 |

$\alpha$ Catenin and E-cadherin expression in gastric carcinomas was classified as ++, +, or − according to the extent of expression. When more than 50%, 50–5%, and <5% of cancer cells were positively stained, the cases were classified as ++, +, and −, respectively.

Table II. Expression of $\alpha$ Catenin and E-Cadherin in Breast Carcinoma

| $\alpha$ catenin expression | | ++ | ++ | ++ | + | + | + | − | − | − |
|---|---|---|---|---|---|---|---|---|---|---|
| E-cadherin expression | | ++ | + | − | ++ | + | − | ++ | + | − |
| Infiltrating ductal carcinoma | | | | | | | | | | |
| Solid type | 14 | 7 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 |
| Scattered type | 8 | 0 | 0 | 0 | 2 | 0 | 0 | 6 | 0 | 0 |
| Infiltrating lobular carcinoma | 12 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 11 |
| Total | 34 | 7 | 0 | 0 | 9 | 0 | 1 | 6 | 0 | 11 |

$\alpha$ Catenin and E-cadherin expression in gastric carcinomas was classified as ++, +, or − according to the extent of expression. When more than 50%, 50–5%, and <5% of cancer cells were positively stained, the cases were classified as ++, +, and −, respectively.

bands of lower molecular weight detected in the Western blotting analysis using $\alpha$ catenin rat monoclonal antibody were thought to be degradation products and nonspecific immunoreaction products based on a comparison with the negative control (Fig. 1A, lane 2). E-Cadherin immunoreactivity was detected at cell membranes from the top to the bottom of whole gastric glands (Fig. 1B). $\alpha$ Catenin immunoreactivity was also detected at the cell membrane in normal gastric mucosa (Fig. 1C). The localizations of $\alpha$ catenin and E-cadherin in the normal epithelial cells were almost identical, but some mucous cells expressed $\alpha$ catenin immunoreactivity in the cytoplasm.

**Immunohistochemistry** The immunohistochemical reactivities of E-cadherin and $\alpha$ catenin in various types of gastric cancer are summarized in Table I. The immunohistochemical staining patterns of E-cadherin and $\alpha$ catenin in various gastric carcinomas are shown in Fig. 2. All 33 cases of differentiated-type adenocarcinomas studied, which comprised papillary and tubular adenocarcinomas, expressed both E-cadherin and $\alpha$ catenin immunoreactivity at the cell-to-cell borders (Fig. 2a, b). In 17 solid undifferentiated-type adenocarcinomas in which cell-to-cell connections were preserved relatively well (Fig. 2c, d), E-cadherin immunoreactivity was detected in all, but 5 (29.1%) were negative for $\alpha$ catenin immunoreactivity. Of the 44 scattered-type undifferentiated gastric adenocarcinomas, 8 (18.1%) had lost E-cadherin and 24 (54.6%) had lost $\alpha$ catenin immunoreactivity (Fig. 2e, f). Among them, 5 were negative for both E-cadherin and $\alpha$ catenin immunoreactivity and 3 were negative for E-cadherin but positive for $\alpha$ catenin (Fig. 2g, h), which showed diffuse cytoplasmic staining. Fig. 2i and j are negative control stainings for E-cadherin (Fig. 2e) and $\alpha$ catenin (Fig. 2h), respectively. No specific immunoreactions were observed in these negative controls. These results indicate that selective down-regulation of $\alpha$ catenin expression occurs in the majority of scattered-type scirrhous gastric adenocarcinomas. This result was consistent with our previous data indicating that many scattered-type undifferentiated adenocarcinomas possess E-cadherin expression, despite their single-cell invasion pattern.

The immunohistochemical reactivities of E-cadherin and $\alpha$ catenin in infiltrating breast carcinomas are

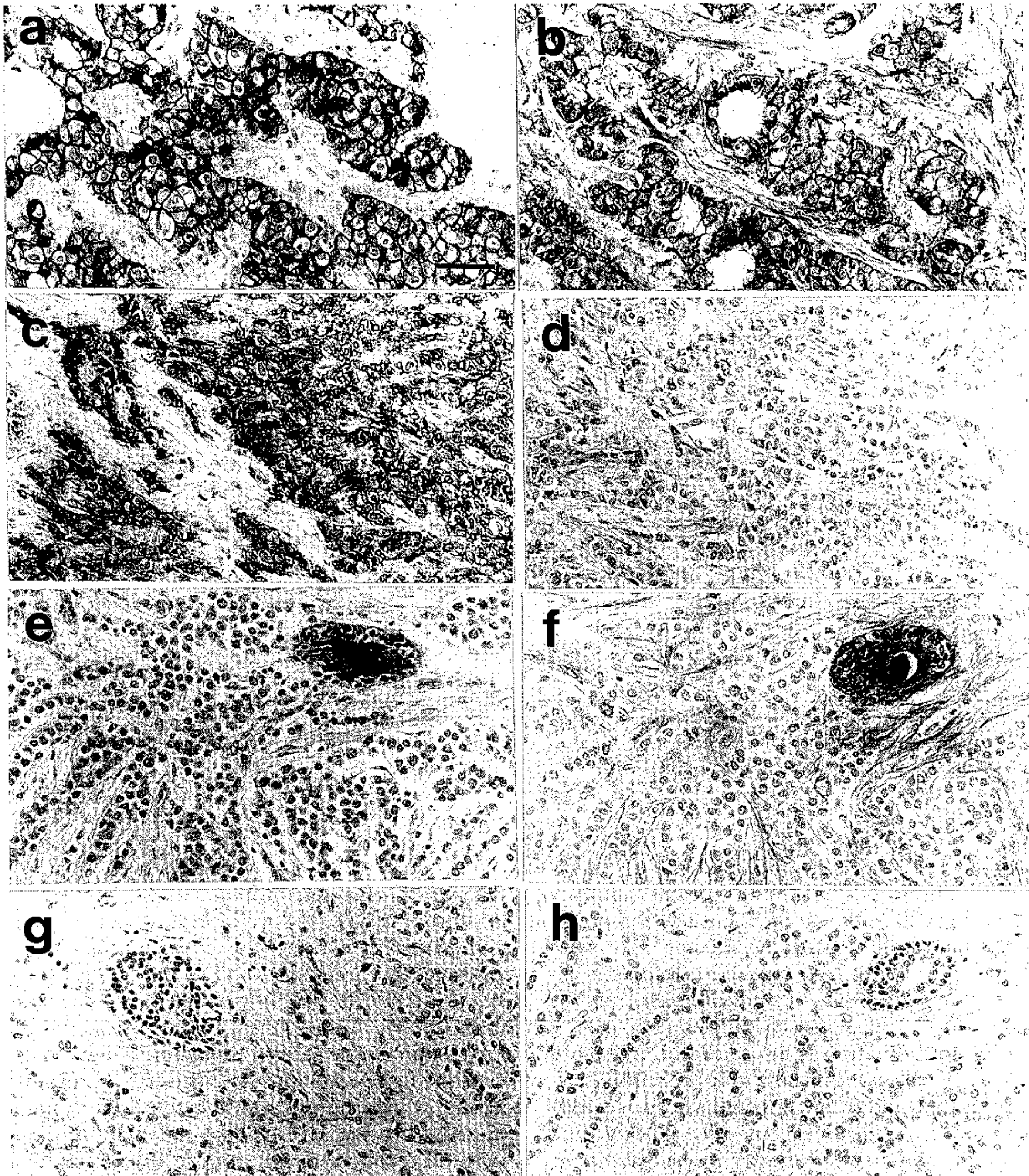


Fig. 3. Immunohistochemical staining of E-cadherin and $\alpha$ catenin in breast carcinomas. Solid-type infiltrating ductal carcinomas (a, b), scattered-type infiltrating ductal carcinomas (c, d, g) and infiltrating lobular carcinomas (e, f, h) were stained with HECD-1 for E-cadherin (a, c, e) and $\alpha$-18 for $\alpha$ catenin (b, d, f) antibodies. Normal mouse (g) and rat immunoglobulin (h) were used for negative control staining. The sections were counterstained with hematoxylin. Scale bar, 20 $\mu$m.

summarized in Table II. All 14 solid-type infiltrating ductal carcinomas studied possessed both E-cadherin and $\alpha$ catenin (Fig. 3a, b). All 8 scattered-type invasive ductal carcinomas were positive for E-cadherin, but 6 (75%) were negative for $\alpha$ catenin (Fig. 3c, d). In contrast to the infiltrating ductal carcinomas, 11 out of 12 infiltrating lobular carcinomas studied exhibited no E-cadherin or $\alpha$ catenin immunoreactivity (Fig. 3e, f)

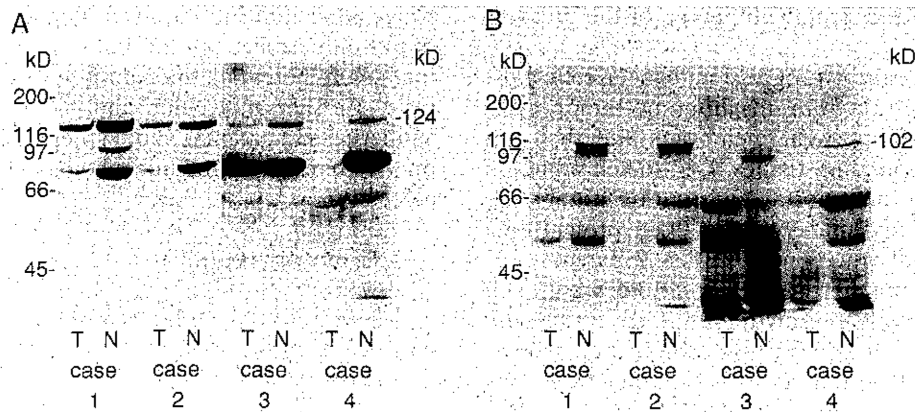


Fig. 4. Western blotting analysis of E-cadherin (A) and α catenin (B) in representative scattered-type undifferentiated adenocarcinomas of the stomach. Lysates loaded on lanes T were extracted from tumor tissues and those on lanes N from corresponding normal tissues. Case 1 is the same as that shown on Fig. 2, e and f.

and one case was E-cadherin negative, but showed focal immunoreactivity for α catenin. Fig. 3g and h are negative control stainings for E-cadherin (Fig. 3c) and α catenin (Fig. 3f), respectively.

**Immunoblotting analysis** The results obtained by immunoblotting analysis were consistent with those by immunohistochemistry. Fig. 4 shows representative immunoblotting analysis patterns of E-cadherin and α catenin expression in scattered-type scirrhous gastric carcinomas. When compared with immunohistochemical studies in cases 1 and 2, expression of HECD-1 was strongly recognized at the cell membrane in cancer cells, but that of α catenin was completely lost. The low E-cadherin expression in these tumor tissues when compared with that of normal tissue demonstrated by immunoblotting can be explained in terms of the small number of cancer cells in scirrhous carcinoma. However, these cancer cells expressed E-cadherin of normal molecular weight (124 kD), whereas the expression of α catenin (102 kD) was completely lost or greatly reduced in some scirrhous adenocarcinomas. In addition, reduced E-cadherin expression and no α catenin expression were observed in case 3, and no expression of either E-cadherin or α catenin was observed in case 4. These data, together with the results of immunohistochemistry in the same tumor demonstrate that selective down-regulation of α catenin occurs frequently in scirrhous carcinoma with scattered cell growth.

## DISCUSSION

Loss of E-cadherin on cancer cells has been reported in squamous cell carcinomas of the head and neck and other organs.[23, 24] The reduction of E-cadherin expression in various cancers may be due to a direct mutation of the E-cadherin molecule. In fact, allele loss on chromosome 16p 22.1 to 23.2, where the E-cadherin gene is located, has been reported in breast cancers.[25] Alternatively, the promoter of these molecules may be repressed at the level of transcription factors.[26] We have reported previously that some cells of gastric and hepatocellular carcinomas that exhibited scattered growth completely lacked cadherin expression.[20, 21, 27] However, we also found that many scirrhous gastric adenocarcinoma cells showing stromal infiltration with scattered cell growth strongly expressed E-cadherin, even though the cells had completely lost their tight cell-to-cell connections.[21] In the present study, selective down-regulation of α catenin expression in scirrhous carcinomas of both the stomach and breast was demonstrated by both immunohistochemistry and immunoblotting analysis. This suggests that the cell-cell disruption observed in scirrhous carcinomas with no organ specificity could be due to loss of α catenin expression. In fact, we found that a lung carcinoma cell line, PC 9, which expressed E-cadherin but not α catenin, had loose cell-to-cell connections. Northern and Southern blotting analyses of this cell line revealed deletion of part of the α catenin gene, and this deletion was concluded to be responsible for cadherin dysfunction.[14, 15]

The complete absence of E-cadherin expression observed in infiltrating lobular carcinomas suggests a fundamental abnormality in such cancer cells. Nagafuchi *et al.*[11] demonstrated the post-transcriptional regulation of α catenin expression by E-cadherin. Therefore the loss of α catenin expression in infiltrating lobular carcinoma of the breast may be attributable to loss of E-cadherin expression. In order to confirm this, further molecular

analysis of lobular and ductal carcinoma of the breast is required.

In the course of cancer progression, cancer cells must first become detached from neighboring tumor cells, invade through the basement membrane and then migrate into the surrounding tissues. Cell-to-cell adhesion molecules may play an important role in this initial stage of cancer invasion and metastasis.[28, 29] Our present data suggest that down-regulation of either $\alpha$ catenin and E-cadherin may cause disruption of cell-to-cell adhesion and initiate the invasive growth of scattered-type undifferentiated carcinomas such as scirrhous and lobular carcinoma. Investigation of cell adhesion molecules may prove important for understanding the basic mechanisms of not only cancer cell morphogenesis but also biological malignant potential, including invasiveness and metastasis. Further molecular and biochemical analyses of cadherins and catenins should yield valuable information on the biological and morphological nature of cancer cells.

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REFERENCES

1) Yoshida-Noro, C., Suzuki, N. and Takeichi, M. Molecular nature of the calcium-dependent cell-cell adhesion system in mouse teratocarcinoma and embryonic cells studied with a monoclonal antibody. *Dev. Biol.*, **101**, 19–27 (1984).
2) Wheelock, M. J., Buck, C. A., Bechtol, K. R. and Damsky, C. H. The soluble GP80 fragment of cell CAM 120/80 disrupts cell-cell adhesion. *J. Cell Biol.*, **110**, 187–202 (1987).
3) Takeichi, M. The cadherins: cell-cell adhesion molecules controlling animal morphogenesis. *Development*, **102**, 639–655 (1988).
4) Shimoyama, Y., Yoshida, T., Terada, M., Shimosato, Y., Abe, O. and Hirohashi, S. Molecular cloning of a human $Ca^{2+}$ dependent cell-cell adhesion molecule homologous to mouse placental cadherin: its low expression in human placental tissues. *J. Cell Biol.*, **109**, 1787–1794 (1989).
5) Takeichi, M. Cadherin cell adhesion receptors as a morphogenetic regulator. *Science*, **251**, 1451–1455 (1991).
6) Tsukita, Sh., Tsukita, Sa. and Nagafuchi, A. The undercoat of adherence junction: a key specialized structure in organogenesis and carcinogenesis. *Cell Struct. Funct.*, **15**, 7–12 (1992).
7) Boeller, K., Vestweber, D. and Kemler, R. Cell adhesion molecule uvomorulin is localized in the intermediate junctions of adult intestinal epithelial cells. *J. Cell Biol.*, **100**, 327–332 (1985).
8) Vestweber, D. and Kemler, R. Some structural and functional aspects of the cell adhesion molecule uvomorulin. *Cell Differ.*, **15**, 269–273 (1984).
9) Nagafuchi, A. and Takeichi, M. Transmembrane control of cadherin-mediated cell adhesion: a 94 kDa protein functionally associated with a specific region of the cytoplasmic domain of E-cadherin. *Cell Regul.*, **1**, 37–44 (1989).
10) Ozawa, M., Baribault, H. and Kemler, R. The cytoplasmic domain of the cell adhesion molecules uvomorulin associates with three independent proteins structurally related in different species. *EMBO J.*, **8**, 1711–1717 (1989).
11) Nagafuchi, A., Takeichi, M. and Tsukita, S. The 102 kD cadherin-associated protein: similarity to vinculin and posttranslational regulation of expression. *Cell*, **65**, 849–857 (1991).
12) Herrenknecht, K., Ozawa, M., Eckerskorn, C., Lottspeich, F., Lenter, M. and Kemler, R. The uvomorulin-anchorage protein $\alpha$ catenin is a vinculin homologue. *Proc. Natl. Acad. Sci. USA*, **88**, 9156–9160 (1991).
13) McCrea, P. D., Turck, C. W. and Gumbiner, B. A homolog of the *armadillo* protein in *Drosophila* (plakoglobin) associated with E-cadherin. *Science*, **254**, 1359–1361 (1991).
14) Shimoyama, Y., Nagafuchi, S., Fujita, S., Gotoh, M., Takeichi, M., Tsukita, S. and Hirohashi, S. Cadherin dysfunction in a human cancer cell line: possible involvement of loss of $\alpha$ catenin expression in reduced cell-cell adhesiveness. *Cancer Res.*, **52**, 5770–5774 (1992).
15) Hirano, S., Komoto, N., Shimoyama, Y., Hirohashi, S. and Takeichi, M. Identification of a neural $\alpha$ catenin as a key regulator of cadherin function and multicellular organization. *Cell*, **70**, 293–301 (1992).
16) Lauren, P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal type carcinoma. *Acta Pathol. Microbiol. Scand.*, **64**, 31–49 (1965).
17) Ming, S. C. "Tumors of the Esophagus and Stomach" (1971). American Registry of Pathology, Armed Forces Institute of Pathology, Washington, D.C.
18) Page, D. L. and Anderson, T. J. "Diagnostic Histopathology of the Breast," pp. 1–362 (1987). Churchill Livingstone, New York.
19) McDivitt, R. W., Stewart, F. W. and Berg, K. W. "Tumors of the Breast" (1968). American Registry of Pathology, Armed Forces Institute of Pathology, Washington, D.C.
20) Shimoyama, Y., Hirohashi, S., Hirano, S., Noguchi, M., Shimosato, Y., Takeichi, M. and Abe, O. Cadherin cell adhesion molecules in human epithelial tissues and carcinomas. *Cancer Res.*, **19**, 2128–2133 (1989).
21) Shimoyama, Y. and Hirohashi, S. Expression of E- and P-cadherin in gastric carcinoma. *Cancer Res.*, **51**, 2185–

2192 (1991).
22) Japanese Research Society for Gastric Cancer. The General Rules for the Gastric Cancer Study. *Jpn. J. Surg.*, **11**, 127–145 (1981).
23) Schipper, J. H., Frixen, U. H., Behrens, J., Unger, A., Jahnke, K. and Birchmeier, W. E-Cadherin expression in squamous cell carcinomas of head and neck: inverse correlation with tumor dedifferentiation and lymph node metastasis. *Cancer Res.*, **51**, 6328–6337 (1991).
24) Doki, Y., Shiozaki, H., Tahara, T., Inoue, M., Oka, H., Iihara, K., Kadowaki, T., Takeichi, M. and Mori, T. Correlation between E-cadherin expression and invasiveness *in vitro* in a human esophageal cancer cell line. *Cancer Res.*, **53**, 3421–3426 (1993).
25) Soto, T., Tanigami, A., Yamakawa, K., Akiyama, F., Kasumi, F., Sakamoto, G. and Nakamura, Y. Allelotype of breast cancer: cumulative allele losses promote tumor progression in primary breast cancer. *Cancer Res.*, **50**, 7184–7189 (1990).
26) Behrens, J., Loewrick, O., Llein-Hitpass, L. and Birchmeier, W. E-Cadherin promoter: functional analysis of a GC-rich region and an epithelial cell-specific palindromic regulatory element. *Proc. Natl. Acad. Sci. USA*, **88**, 11495–11499 (1991).
27) Shimoyama, Y. and Hirohashi, S. Cadherin intercellular adhesion molecule in hepatocellular carcinomas: loss of E-cadherin expression in an undifferentiated carcinoma. *Cancer Lett.*, **57**, 131–135 (1991).
28) Frixen, U. H., Behrens, J., Sachs, M., Eberle, G., Voss, B., Warda, A., Loechner, D. and Birchmeier, W. E-Cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. *J. Cell Biol.*, **113**, 173–185 (1991).
29) Vleminckx, K., Vakaet, L., Jr., Mareel, M., Fiers, W. and VanRoy, F. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. *Cell*, **66**, 107–119 (1991).