Expression of E- and P-Cadherin in Gastric Carcinomas

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ABSTRACT

The expression pattern of two Ca\(^{2+}\)-dependent intercellular adhesion molecules, E- and P-cadherin, in 54 surgically resected gastric adenocarcinomas was examined immunohistochemically. E-cadherin was expressed uniformly at the cell-cell borders of most of the differentiated and adenotype-undifferentiated gastric adenocarcinomas, showing that E-cadherin serves as the main cadherin molecule responsible for intercellular binding in these carcinomas. Scattered-type undifferentiated gastric adenocarcinomas which apparently lacked this tight intercellular adhesion were divisible into two groups on the basis of E-cadherin expression. In a minor group composed of 4 carcinomas, E-cadherin could not be detected, suggesting that the absence of E-cadherin made the cancer cells separate. In contrast, cancer cells of 19 carcinomas which belonged to the major group showed similar scattering but had definite expression of E-cadherin on their cell surfaces, suggesting that there was some mechanism(s) disturbing the function of E-cadherin in these carcinomas. However, immunoblotting showed no evidence of gross alterations of the E-cadherin molecule, such as partial deletion, in these carcinomas. P-cadherin was expressed in 29 (54%) of the examined gastric carcinomas, and the expression was unstable in most of them, a characteristic feature compared with the stable expression of E-cadherin. Since P-cadherin is known to be expressed temporarily in the foregut during development of electron microscopic techniques, it was revealed that the mutual adhesiveness of cancer cells is significantly weaker than that of the corresponding normal cells (1-3), and this reduced adhesiveness was considered to promote the invasive capacity of cancer cells (4, 5). Thereafter, with the development of electron microscopic techniques, it was revealed that deficiencies of intercellular junctional structures are quite common in various malignant tumors (6). In this context, it is of considerable interest and importance to clarify the mechanism responsible for the reduced mutual adhesiveness of cancer cells and its influence on their biological behavior. A number of molecules involved in the intercellular adhesion of epithelial cells have been identified over the last decade (7). These cell adhesion molecules may be divided into two groups, calcium-dependent ones, termed "cadherins" (8), and calcium-independent ones. Cadherins are known to mediate tighter and stronger intercellular adhesion accompanied by cell deformation than calcium-independent adhesion molecules (9) and to be involved in the formation of some junctional structures (10). Cadherins constitute a gene family composed of at least three subclasses, E-, N-, and P-cadherin, which mediate cell-cell binding in a homophilic and subclass-specific manner (11). E-cadherin is expressed by almost all epithelial cells and is considered to be the main cadherin type responsible for their intercellular adhesion (12). E-cadherin is also called uvomorulin (13), cell-CAM 120/80 (14), or Arc-1 (15). L-CAM (16) may be chicken E-cadherin. N-cadherin is expressed in neural tissue and muscle (17-19), but continuous expression of N-cadherin in epithelial tissues has not been reported. P-cadherin was originally identified in mouse placental tissue as a molecule which appeared to act as a connector between the embryo and the uterus (20, 21).

Studies of cadherins have been carried out mainly in the field of developmental biology using experimental animals and have revealed that many morphogenetic events in the embryo are correlated with a unique spatiotemporal pattern of cadherin expression (11). However, since few studies of human cadherins have been done until recently, the expression and role of cadherins in human tissues are not known in detail. In a previous paper we reported the establishment of two mAbs, \(^4\)HECD-1 and NCC-CAD-299, which are immunohistochemically specific for E- and P-cadherin in human tissues, respectively, and also the distribution of E- and P-cadherin in normal human tissues (12). The expression pattern of E-cadherin in humans corresponded well to that in tissues of other animals, whereas that of human P-cadherin was different from that of mouse in one respect: it was not detected in human placental tissue. This finding raised the possibility that the molecule was not P-cadherin itself but P-related cadherin. To settle this issue, we carried out cloning and sequencing of a complementary DNA-encoding human P-cadherin and demonstrated that the molecule was, in fact, authentic human P-cadherin (22). In another respect, P-cadherin showed a very unique distribution in human tissues: although it was detected only in epithelial tissues in a similar manner to E-cadherin, its distribution was restricted to the basal or lower layers of stratified epithelia, suggesting a close relationship between P-cadherin and cell proliferation. This finding prompted us to examine P-cadherin expression in cancer tissues in comparison with that in the corresponding normal tissues.

We also observed in our previous studies that cultured cells forming colonies became motile after they had been dissociated by addition of anti-cadherin mAbs. This suggested the possibility that cancer cells deprived of their cadherin function might acquire the capacity to invade surrounding tissues through their own ameboid motion. Recently, Behrens et al. (23) showed that this possibility is quite likely. In their paper they described that epithelial cells deprived of their cadherin function by addition of anti-cadherin antibodies became able to invade collagen gels and embryonal heart tissue.

In addition to their invasiveness, cancer cells possess another biological characteristic, the ability to metastasize. Reduced...

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\(^4\) The abbreviations used are: mAb, monoclonal antibody; HNC, a buffer containing 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.4), 150 mM NaCl, and 2 mM CaCl\(_2\).
intercellular adhesion might be considered to promote metastasis because this could facilitate the invasion of cancer cells into vessels and the detachment of cancer cells from any tumor protruding into the vessels. Unstable expression of cadherin in a highly metastatic ovarian tumor line has been reported (24), but it is still obscure whether metastasis is affected by cadherin function.

In these respects, it is very important clinically to know the pattern of cadherin expression in cancer tissues. In this paper we report the expression of E- and P-cadherin in surgically resected gastric carcinomas determined using immunohistochemistry, and we discuss the possible roles of cadherins responsible for the biological properties of cancer cells. We chose gastric carcinomas for this study because P-cadherin is scarcely detectable in the corresponding normal cells and because gastric carcinomas which almost completely lack adhesion between the cancer cells are often encountered.

MATERIALS AND METHODS

Surgical Specimens. Fresh cancer tissues as well as individual noncancerous tissues were obtained at the time of surgery in the National Cancer Center Hospital from 54 primary gastric carcinomas. The specimens were fixed with 2% paraformaldehyde in HNC at 4°C for 1 h, followed by infiltration with a graded series of sucrose (10–20%) in HNC at 4°C. They were then embedded in OCT compound (Miles, Elkhart, IN) and frozen on a sheet of copper at −80°C. Frozen sections were cut on a cryostat at a thickness of 6 μm, mounted on poly-lysine-coated slides, and air dried. The sections were used immediately or stored at −80°C until use.

Antibodies. The establishment and specificity of mAbs, HECD-1 and NCC-CAD-299, which recognize human E- and P-cadherin, respectively, have been described previously (12). These antibodies were obtained from ascitic fluid of mice in which specific hybridoma cells had been inoculated i.p., diluted, and used for immunohistochemistry and immunoblotting.

Immunohistochemistry. Immunohistochemistry was performed using serial sections at room temperature unless otherwise mentioned. The sections were postfixed with 2% paraformaldehyde in HNC at 4°C for 10 min and then immersed in 0.3% H2O2 in absolute methanol for 1 h to inhibit endogenous peroxidase activity. The sections were rinsed with HNC three times and treated with 2% normal swine serum in HNC for 30 min to reduce nonspecific staining. They were subsequently incubated with NCC-CAD-299 or HECD-1 mAbs diluted 1/500 for 2 h. Then they were incubated with biotinylated anti-mouse IgG (Vector Laboratories Inc., Burlingame, CA) diluted 1/200 for 30 min, followed by a 30-min incubation with a 1/100 dilution of avidin-biotin-peroxidase complex (Elite ABC, Vector Laboratories). These steps were separated by rinsing with HNC three times and successive brief incubation with 2% normal swine serum in HNC. Antibodies and avidin-biotin-peroxidase complex were diluted with 2% normal swine serum in HNC. Finally, the sections were rinsed thoroughly with HNC and stained for 2–3 min with 0.2 mg/ml dianminobenzidine in 50 mM Tris-HCl (pH 7.6) containing 0.006% H2O2. The sections were counterstained with hematoxylin, dehydrated, and mounted.

Immunohistochemical Criteria of Cadherin Expression. When >90% of the carcinoma cells were positively stained, the case was regarded as uniformly positive, ++. When 5–90 and <5% of carcinoma cells were positively stained, the cases were regarded as partially positive, +, and negative, −, respectively. Any carcinoma showing very weak staining which was difficult to distinguish from the background level was regarded as negative even if all of the carcinoma cells were stained. These criteria were used for judgment of the expression of both E- and P-cadherin.

Histopathological Grading. As well as sections used for immunohistochemistry, an additional serial section in each case was stained with hematoxylin and eosin to determine the histopathological grading, which was defined in this study as follows. Gastric carcinomas were first classified into differentiated-type adenocarcinomas forming papillary and/or tubular structures and undifferentiated-type adenocarcinomas in which such structures were inconspicuous. Signet ring cell carcinomas were classified as undifferentiated. Undifferentiated gastric adenocarcinomas were further divided into two groups, adherent type and scattered type. The former included cases in which the cancer cells showed tight mutual adhesiveness, whereas undifferentiated adenocarcinomas lacking tight intercellular adhesion between the cancer cells were placed in the latter category.

RESULTS

E- and P-Cadherin in Noncancerous Epithelia of the Stomach. As shown in Fig. 1A, E-cadherin was strongly expressed in gastric epithelium from the surface to deep glands without exception. P-cadherin was not detected in almost all of the gastric epithelia examined, although in part of them weak staining of P-cadherin was occasionally observed at the bases of the pits and the tops of the glands (Fig. 1B), which are known to be the proliferative zone of the human gastric epithelium (26, 27).

E- and P-Cadherin in Gastric Carcinomas. As summarized in Table 1, gastric carcinomas examined here consisted of 17 differentiated and 37 undifferentiated adenocarcinomas. Twenty-eight of the undifferentiated adenocarcinomas contained scattered-type lesions (Table 1). There were no special-type carcinomas.

All of the differentiated adenocarcinomas expressed E-cadherin uniformly and strongly without exception, as did noncancerous gastric epithelia (Table 1; Fig. 2, A, C, and E). On the other hand, P-cadherin was expressed in 15 of the 17 differentiated adenocarcinomas (88%) (Table 1; Fig. 2, B, D, and F). In 13 of the P-cadherin-positive cases, carcinomatous lesions were composed of a mixture of cancer cells expressing or not expressing P-cadherin (Fig. 2D), suggesting instability of P-cadherin expression in these carcinomas.

As can be seen in Fig. 3, A, C, and E, adherent-type undifferentiated adenocarcinomas expressed E-cadherin uniformly and strongly, as did differentiated adenocarcinomas except for one case which showed unstable expression of E-cadherin (Table 1). In contrast, E-cadherin was not detected in 4 of the scattered-
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Fig. 1. Immunohistochemical detection of E- and P-cadherin in noncancerous gastric epithelium in the pyloric region. A and B, stained for E-cadherin and P-cadherin, respectively; arrowheads, weak staining for P-cadherin. Scale bar, 100 µm.

Table 1 Expression of E- and P-cadherin in gastric carcinomas

<table>
<thead>
<tr>
<th></th>
<th>E-cadherin expression</th>
<th>P-cadherin expression</th>
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<tr>
<td></td>
<td>++ ++ ++ ++ + + + + -</td>
<td>++ + - ++ + - ++ + -</td>
</tr>
<tr>
<td>Differentiated-type adenocarcinomas (17)</td>
<td>2 13 2 0 0 0 0 0 0</td>
<td>2 8 18 1* 2 2 0 1 3</td>
</tr>
<tr>
<td>Undifferentiated-type adenocarcinomas (37)</td>
<td>2 1 3 15 0 2 2 0 1 3</td>
<td></td>
</tr>
<tr>
<td>Scattered-type adenocarcinomas (28)</td>
<td>1 2 1 3 0 1 3</td>
<td></td>
</tr>
<tr>
<td>Total (54)</td>
<td>4 21 20 1 2 2 0 1 3</td>
<td></td>
</tr>
</tbody>
</table>

* Cadherin expression in gastric carcinomas was classified as ++, +, or − according to the extent of expression. When more than 90%, 90-5%, and <5% of cancer cells were positively stained, the cases were regarded as ++, +, and −, respectively.

* Values in parentheses, numbers of cases.

* This was the only adherent-type carcinoma which showed unstable expression of E-cadherin.

* Twenty-eight of 37 undifferentiated adenocarcinomas contained the scattered-type lesion in which tight intercellular adhesion between cancer cells could not be seen. Pattern of cadherin expression in these lesions is also indicated.

We also examined the histopathological features and cadherin expression of lymph node metastases of 20 cases in comparison with those of each primary tumor. In all of the metastases, E-cadherin was expressed at the same intensity and extent as in the individual primary carcinomas (Fig. 6A). Cancer cells which had invaded lymphatic vessels also expressed E-cadherin at the same intensity and extent (Fig. 6B). With regard to P-cadherin, there were three cases in which the expression in metastasis was less extensive than that in the primary tumor (data not shown). However, there were also two cases in which P-cadherin expression in metastasis was more extensive than that in the primary tumor (data not shown). These findings probably suggest that P-cadherin expression was unstable in the metastases as well as in the primary tumors.

DISCUSSION

E-cadherin is expressed in most human epithelial tissues and is thought to be the main cadherin molecule responsible for the maintenance of epithelial architecture (12, 28). In this study also, E-cadherin was shown to be expressed in most of the gastric adenocarcinomas examined and possibly to function as the main cadherin molecule in these malignancies. However, we also encountered a few carcinomas which apparently lacked E-cadherin expression immunohistochemically (4 of 54 gastric adenocarcinomas). It was noteworthy that all 4 of these carcinomas appeared to lack intercellular adhesion between cancer cells. Taking these findings together with the previous experimental demonstration of a correlation between cadherin expression and intercellular adhesiveness in transfection experiments...
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Fig. 2. Immunohistochemical detection of E- and P-cadherin in differentiated gastric adenocarcinomas. A, C, and E, stained for E-cadherin; regarded as ++ for E-cadherin expression. B, D, and F, stained for P-cadherin; serial sections of A, C, and E, respectively; regarded as ++, +, and −, respectively, for P-cadherin expression. Scale bar, 100 μm.

(22, 29) and a correlation between removal of cadherin function and acquisition of invasive capacity (23), it is quite reasonable to consider that loss of E-cadherin expression or function frees carcinomas from tight intercellular adhesion and possibly enhances their invasiveness.

On the other hand, approximately two thirds of the scattered-type undifferentiated gastric adenocarcinomas lacking tight intercellular adhesion expressed E-cadherin uniformly and strongly on the surfaces of the cancer cells. To account for this phenomenon, several possible mechanisms can be considered, such as alterations of the E-cadherin molecule itself and extrinsic or intrinsic factors interfering with the binding function of E-cadherin. Immunoblotting of the scattered carcinomas showed that they did express the complete and functional form of the molecule, as did noncancerous epithelia showing tight intercellular adhesion, although the existence of tiny modifications which had abolished the function but did not influence the reactivity with mAb HEC-1 and mobility on sodium dodecyl sulfate-polyacrylamide gel electrophoresis could not be ruled out. Some unknown factor(s) increasing the motility of cancer cells and inhibiting the formation of intercellular adhesion might be involved in the development of scattered carcinomas. Such a factor, called a “scatter factor,” has been reported but was found not to be effective on cancer cells (30).
Intrinsic molecules which anchor cadherin to the cytoskeleton and/or mediate the movement of cadherin on the cell surface to the site of cell-cell binding are the most noteworthy factors for which aberrations might render cadherin ineffective. In fact, association of the cytoplasmic domain of the cadherin molecule with the cytoskeleton is known to be indispensable for the expression of cell-cell binding function (31), and some candidate molecules which may anchor cadherin to the cytoskeleton have been reported (32, 33). Determination of whether aberrations of these molecules really do cause the malfunction of cadherin, make cancer cells dissociate from one another, and render them more invasive is considered to be one of the most important tasks for the future.

We were unable to obtain any evidence that scattered-type cancer was more metastatic than the adherent type or that down-regulation of cadherin expression was involved in the process of metastasis to regional lymph nodes, although the disruption of cell-cell contact is considered to be a prerequisite for metastasis. However, there may be a possible explanation for this. Even when cancer cells are bound to one another, it has been shown that their mutual adhesiveness is significantly weak (1–3) and that their development of junctional structures.
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Fig. 4. Immunohistochemical detection of E-cadherin in scattered-type undifferentiated gastric adenocarcinomas. A, a signet ring cell carcinoma classified as a scattered-type undifferentiated gastric adenocarcinoma. Cancer cells lie mainly on the right of the figure, and some of them are indicated by arrowheads. This case is regarded as — for E-cadherin expression. Note that noncancerous epithelia (N) on the left clearly express E-cadherin at the cell-cell border (arrows). B, a signet ring cell carcinoma classified as the adherent type. This case is included for comparison with A. Carcinoma cells express E-cadherin (+++) at the cell-cell border (arrows) and form tight intercellular adhesion. C and D, scattered-type undifferentiated gastric adenocarcinomas expressing E-cadherin (++). Scale bar, 50 μm.

is frequently insufficient (6). It is conceivable that the weak mutual adhesiveness between cancer cells, which probably cannot be explained only in terms of the intensity of cadherin expression, plays an indispensable role in the accomplishment of metastasis. However, as described in the above paragraph, the possibility that some malfunction(s) of cadherin may be responsible for the weak mutual adhesiveness still remains to be investigated.

We reported previously that the distribution of P-cadherin is restricted to the basal or lower layers of stratified epithelia (12). In that study we were unable to detect P-cadherin in simple epithelia, such as gastrointestinal epithelia. However, close and extensive immunohistochemical examination disclosed that part of the gastric epithelium defined as the proliferative zone occasionally expressed P-cadherin, although weakly, suggesting that P-cadherin is a cell adhesion molecule which may be expressed in cells having high growth activity and/or high differentiation potential, thus increasing our interest in P-cadherin expression in gastric carcinomas. In fact, 29 (54%) of the examined gastric carcinomas showed definite expression of P-cadherin, although further studies must be done before it can be concluded that P-cadherin is directly correlated with the proliferative ability of gastric carcinomas. Interestingly, it has also been reported that P-cadherin is expressed in the foregut at the neurulation stage in mouse embryos (20). It can be concluded from these findings that P-cadherin is a member of...
the oncofetal protein family, which also includes carcinoembrionic antigen (34) and α-fetoprotein (35, 36).

Recently, some implications of intercellular adhesion molecules in cancer have been elucidated by different approaches. The cluster 1 antigen, which is known to appear specifically in small cell lung carcinomas (37, 38), has been proved to be a human neural cell adhesion molecule (39). Carcinoembryonic antigen has also been demonstrated to be homologous to neural cell adhesion molecule and other members of the neural cell adhesion molecule family (37, 38). The DCC gene, a candidate for a tumor-suppressor gene located on chromosome 18q and involved in colorectal carcinogenesis, has also been proved to have significant sequence similarity to neural cell adhesion molecule and other members of the DCC gene family (47). However, these molecules all belong to the immunoglobulin superfamily and mediate intercellular adhesion in a Ca²⁺-independent manner (although the function of the DCC gene product is still unclear). The need is becoming increasingly apparent for more detailed investigation of cadherins, which mediate tighter and stronger adhesion than Ca²⁺-independent molecules (9), in various malignant neoplasms.

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REFERENCES


Fig. 6. Immunohistochemical detection of E-cadherin in a lymph node metastasis (A) and clusters of cancer cells in a lymphatic vessel (B). E-cadherin expression was ++ in both cases, as in the individual primary tumors. The primary lesion of A is shown in Fig. 3A. Scale bar, 100 μm.


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