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Short Communication

β-Catenin Expression in Human Cancers

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Cell-cell adhesion in tissue is mainly regulated by homotypic interaction of cadherin molecules, which are anchored to the cytoskeleton via cytoplasmic proteins, including α- and β-catenin. Although we previously demonstrated that α-catenin is crucial for cadherin function* in vivo*, little is known about the role of β-catenin. We examined the expression of β-catenin in human carcinoma samples along with normal tissue (esophagus, stomach, and colon) by immunostaining using our antibody for β-catenin. Normal epithelium strongly expressed β-catenin. However, β-catenin expression was frequently reduced in primary tumors of the esophagus (10 of 15, 67%), stomach (9 of 19, 47%), and colon (11 of 22, 50%). From an immunoprecipitation study, we found that β-catenin forms a complex with E-cadherin not only in the normal epithelium but also in cancerous tissues. In coexpression patterns of E-cadherin and β-catenin, 43 (77%) of the 56 tumors showed a similar expression of both molecules, whereas the other 13 tumors (23%) showed positive staining for E-cadherin and reduced expression of β-catenin. These findings suggest that β-catenin forms a complex with E-cadherin* in vivo *and down-regulation of β-catenin expression is associated with malignant transformation. (Am J Pathol 1996, 148:39–46)

Cadherin, a cell-cell adhesion molecule, contains a transmembrane glycoprotein that accounts for its homotypic adhesion in the presence of calcium and plays an important role in the organization and maintenance of tissue structure.[1,2] Normal epithelial cells tightly bind to each other, whereas cancer cells exhibit a looser association and invasive behavior. We previously demonstrated that E-cadherin expression is completely or heterogeneously impaired in a significant proportion of carcinomas and that its reduction was significantly correlated with the degree or differentiation, invasiveness, and metastasis.[3–6] The impaired function of E-cadherin appears to be important in the acquisition of invasive potential. Cadherins including E-cadherin are associated with a group of catenins, including α-catenin (102 kd) and β-catenin (95 kd), which act to couple the cadherins with the microfilament of the cytoskeleton.[7–9] α-Catenin is homologous to vinculin, which is localized in adherens junction and in focal contact. We previously showed that the adhesion system in cancers mediated by E-cadherin is affected by reduced expression of α-catenin and also suggested that α-catenin may affect the invasive or metastatic behavior of cancer cells.[10–12]

From sequence analysis of cDNA, β-catenin shows homology to plakoglobin, a major component of desmosomes and adherens junctions, and to armadillo, a *Drosophila* segment polarity gene.[13] A recent study using the *Xenopus* embryogenesis system reported that the antibody for β-catenin induces a secondary body axis and also suggested that β-catenin is responsible for organogenesis and morphogenesis of tissues.[14] β-catenin is tyrosine phosphorylated in v-*src*-transformed

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cells and this process is correlated with cell transformation.[15–17] Recently, we also demonstrated that tyrosine phosphorylation of β-catenin might modulate or suppress the function of the cadherin-mediated cell adhesion system.[18,19] Furthermore, β-catenin has been reported to interact with the adenomatous polyposis coli (APC) suppressor gene product[20,21] and to induce signal transduction through epidermal growth factor.[18,19,22] It was also suggested that mutation of APC might render inadequate binding capacity between APC and β-catenin. This interesting association between these factors *in vivo* is not yet well understood. Thus, from these biological behaviors, it is of interest to investigate the expression of β-catenin in normal and cancerous tissues *in vivo*.

In this study, we examined the expression of β-catenin in primary cancer tissues (esophagus, stomach, and colon) by immunohistochemical techniques using our specific antibody for β-catenin and evaluated its possible correlation with E-cadherin and clinicopathological factors.

Materials and Methods

Patients

Fifteen patients with squamous cell carcinoma of the esophagus, nineteen with adenocarcinoma of the stomach, and twenty-two with adenocarcinoma of the colon who underwent surgery at our department were included in this study. None of them received anti-cancer therapy preoperatively. Normal epithelial tissues were obtained from noncancerous mucosa near the tumor in all the patients examined.

Antibodies

A keyhole limpet hemocyanin-conjugated synthetic peptide corresponding to the COOH-terminal 14 amino acids (PGDSNQLAWFDTDL) of human β-catenin was directly used as an antigen. A rabbit polyclonal antiserum to β-catenin was raised by immunization with this synthetic peptide by standard procedures, as described previously. The specificity of this antibody was identified; a 95-kd band, corresponding to β-catenin, was detected in total cellular lysates by immunoblotting analysis with this antibody.[23] A mouse monoclonal antibody (HECD-1) against human E-cadherin was purchased from Takara Shuzo Co. (Kyoto, Japan). This antibody recognizes an extracellular epitope of the E-cadherin. The specificity of this antibody was described previously.[24]

Immunohistochemistry

The fresh samples were embedded in optimal cutting temperature (OCT) compound (Miles Laboratories, Elkhart, IN) and immediately frozen by dry ice acetone. The avidin-biotin-peroxidase complex (ABC) technique was used for immunohistochemical staining as reported previously.[25] In brief, the 4-μm-thick frozen sections were made by a cryostat, fixed with 3.6% paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.4), treated with 1% hydrogen peroxide in methanol for 30 minutes to inhibit endogenous peroxidase, and washed with 0.01 mol/L, pH 7.2, Tris buffer. After incubation with 3.0% normal goat serum or 3.0% normal horse serum to block nonspecific binding, the sections were incubated with the primary antibody against β-catenin or E-cadherin at 4°C overnight with biotinylated anti-rabbit IgG or biotinylated anti-mouse IgG (Vectastain ABC kit, Vector Laboratories, Burlingame, CA) for 30 minutes at room temperature and with ABC reagent (Vectastain ABC kit) for 30 minutes. The color was developed with diaminobenzidine supplemented with 0.02% hydrogen peroxide for 4 minutes and counterstained with Mayer's hematoxylin (Chroma-Gesellschaft, Schmid GMBH and Co., Stuttgart, Germany). Normal epithelium or gland included in the samples were treated as an internal positive control. Negative controls consisted of adjacent sections in which the primary antibody was replaced with a nonimmune rabbit serum or mouse IgG1 in which the primary antibody was raised.

Evaluation of Immunostaining

In cancerous tissue, the intensity of β-catenin and E-cadherin were evaluated semiquantitatively using normal epithelial cells in the same section as an internal positive control. If the intensity in tumor cells was the same as in normal epithelial cells, the tumor cells were evaluated as preserved expression (+). In cases in which the intensity of staining was homogeneously weak or variable, the cancerous tissue was evaluated as reduced expression (±). The sections adjacent to the immunohistochemically analyzed ones were stained with hematoxylin and eosin for histological evaluation. Assessment of the staining was carried out using light microscopy by at least two independent observers with no knowledge of histological grade and patient's history.

Immunoblotting

To confirm our immunohistochemical staining, we performed immunoblot analysis on the representative samples of gastric cancer in each staining type as described previously. In brief, tissue fragments were dissolved in a buffer containing 2% sodium dodecyl sulfate (SDS). After being boiled for 5 minutes in the presence of 2-mercaptoethanol, the resulting lysate were separated by SDS polyacrylamide gel electrophoresis using 7.5% polyacrylamide gels. After electrophoresis, proteins were transferred to a nitrocellulose membrane, incubated with the primary antibody against β-catenin, and visualized with a blotting detection kit (Bio-Rad, Hercules, CA). Total cellular proteins applied to each lane were measured with a Bio-Rad protein assay kit and adjusted to equal concentrations.

Immunoprecipitation

For immunoprecipitation of E-cadherin, gastric mucosa and gastric cancer tissues were homogenized in the extraction buffer (0.5% Nonidet P-40, 2 mmol/L $CaCl_2$, 2 mmol/L phenylmethylsulfonyl fluoride, and 20 μg/ml leupeptin in 50 mmol/L Tris buffer, pH 7.4) and centrifuged at 15,000 rpm for 20 minutes at 4°C. The supernatant was preabsorbed by incubation with 50 μl of protein A Sepharose (Pharmacia LKB Biotechnology, Uppsala, Sweden) for 30 minutes. After removing the beads, the lysate containing 200 μg of protein was mixed with monoclonal antibody against E-cadherin (HECD-1) for 2 hours and then incubated with the protein A Sepharose for 2 hours. The beads were collected by centrifugation, washed five times with the extraction buffer, and suspended in SDS sample buffer with 5% 2-mercaptoethanol, and boiled for 5 minutes. The released materials were analyzed by immunoblotting.

Results

Immunostaining

Normal squamous epithelium of esophagus showed strong β-catenin expression especially on cell-cell boundaries except in the superficial layer. All of the normal specimens examined expressed uniformly positive staining for β-catenin (Figure 1A). Noncancerous epithelial cells of gastric crypts and glands strongly expressed β-catenin homogeneously on cell-cell boundaries without exception (Figure 1D). We also obtained the same findings in normal colorectal epithelium (Figure 1G). Endothelium and smooth muscle cells were stained faintly positive but clearly weaker than in normal epithelial cells. Blood cells and lymphocytes did not express β-catenin at all (data not shown).

Of the 15 esophageal cancers, 5 (33%) were evaluated as β-catenin preserved (+) and 10 (67%) were evaluated as reduced expression (±) (Figure 1, B and C). Of the 19 gastric cancers examined, 10 (53%) and 9 (47%) tumors were classified as (+) and (±) for β-catenin expression, respectively (Figure 1, E and F). In 22 colorectal cancers examined, 11 (50%) and 11 (50%) were evaluated as (+) and (±) for β-catenin expression, respectively (Figure 1, H and I). We could not find any case that showed complete loss of expression of β-catenin in this study.

In the β-catenin (+) tumors, cancer cells express β-catenin on the cell-cell boundaries same as normal epithelial cells (Figure 1, B, E, and H). On the other hand, in the β-catenin (±) tumors some showed obscure or diffuse staining in the cytoplasm (Figure 1, C, F, and I). Negative control sections showed no staining (data not shown).

Immunoblot and Immunoprecipitation Analysis

Figure 2A shows the immunoblot analysis of β-catenin in normal gastric mucosa and representative cases of two expression patterns in cancers. Immunoblotting of normal gastric mucosa revealed the darkest band at the molecular mass of 95 kd, which corresponded to the complete form of the β-catenin molecule. By our criteria, β-catenin (+) tumor expressed the 95-kd band as strong as normal epithelium, whereas β-catenin (±) tumors were weaker. Thus, the grade of β-catenin expression determined by immunohistochemical evaluation correlated with those determined by immunoblot analysis.

To reveal whether β-catenin forms a complex with E-cadherin in human cancerous tissues, we immunoprecipitated E-cadherin from lysates of normal gastric mucosa or gastric cancer tissues and examined the co-precipitated proteins with E-cadherin by immunoblot analysis of β-catenin (Figure 2B). In normal gastric mucosa (lane 3) and β-catenin (+) tumor (lane 4), the strong bands at 95 kd were observed. A weak band also appeared in β-catenin (±) tumor (lane 5). These results indicated that β-catenin forms a complex with E-cadherin both in normal epithelium and cancerous tissues.

We also obtained the same findings in other cancers (esophagus and colon; data not shown).

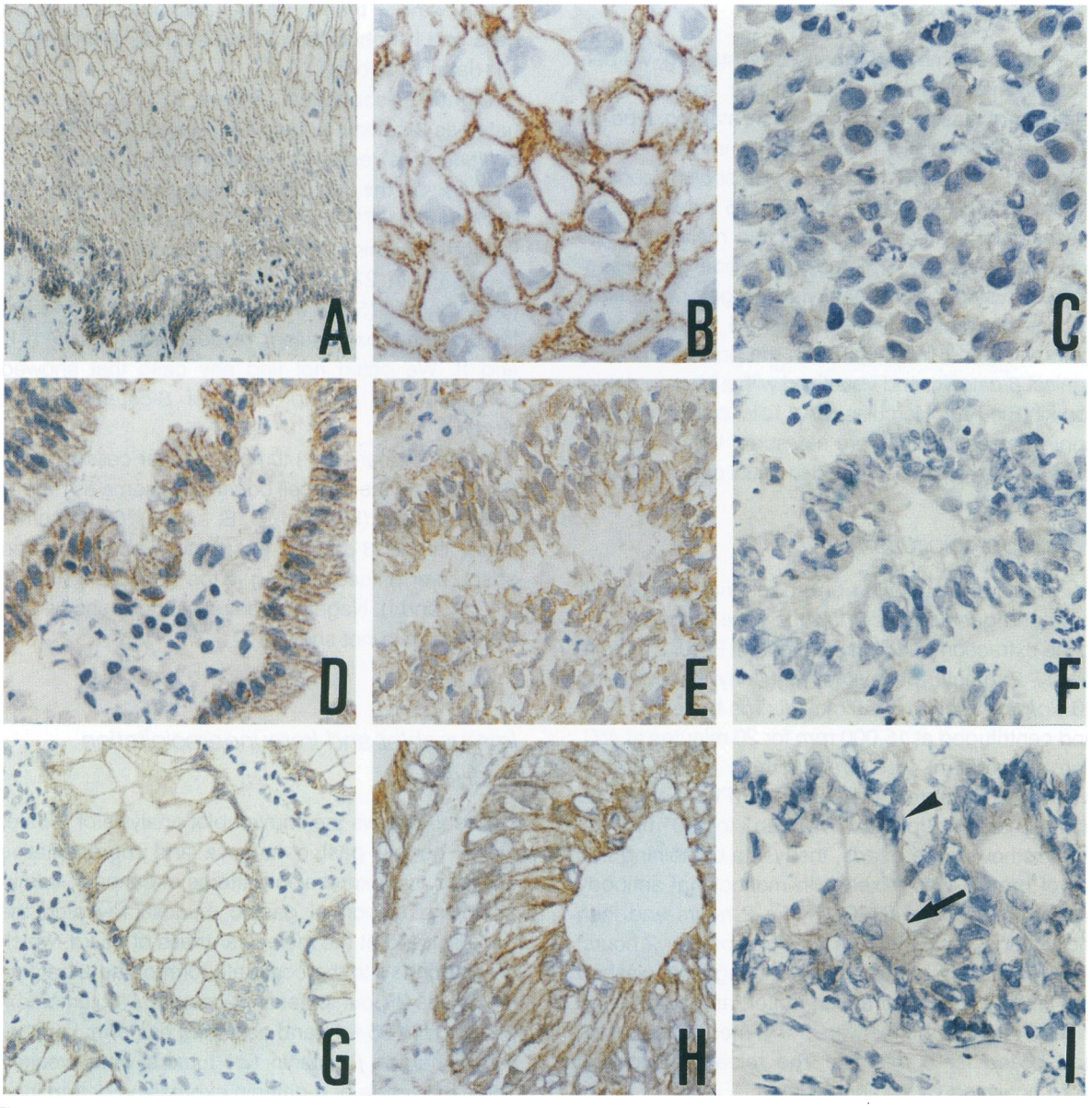

Figure 1. *Immunoreactivity of β-catenin expressions in normal epithelium and cancers.* A: *Normal esophageal squamous epithelium.* B *and* C: *Esophageal squamous cell cancer.* B: *All of the tumor cells express on cell-cell boundaries the same as noncancerous epithelial cells. This tumor was classified as β-catenin (+).* C: *All of the tumor cells express homogeneously weak expression. This tumor was classified as β-catenin (±).* D: *Normal gastric glands.* E *and* F: *Adenocarcinoma of stomach.* E *and* F *were evaluated as β-catenin (+) and (±), respectively.* G: *Noncancerous crypts of colon.* H *and* I: *Adenocarcinoma of colon.* H *and* I *were evaluated as β-catenin (+) and (±), respectively.* I *In the β-catenin (±) tumor, variable staining was observed, in some areas preserved and other areas reduced.* Arrow *and* arrowhead *indicate the β-catenin-positive and reduced cells, respectively. Original magnification, ×66 (*A*) and ×100 (*B *to* L*).*

β-Catenin Expression and Histological Grade

Table 1 shows the relationship between β-catenin expression and histological grade. β-Catenin (±) tumors were observed more frequently in poorly differentiated carcinoma than in well differentiated carcinoma. In colorectal cancer, we found significant correlation between histological grade of the tumors and reduction in β-catenin expression. However, this significant correlation was not observed in esophageal or gastric cancer.

Coexpression of E-Cadherin and β-Catenin

Results of coexpression pattern of E-cadherin and β-catenin (E-cad/β-cat) are shown in Table 2. The 55 esophageal cancers were classified as follows: 5 (33%) E-cad+/β-cat+, 3 (20%) E-cad+/β-cat±, and

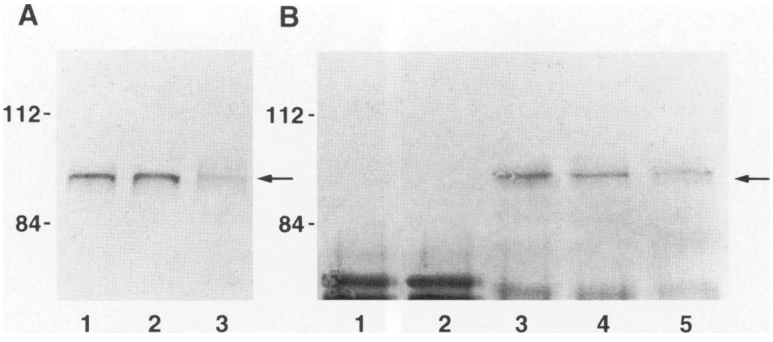

Figure 2. A: *Immunoblot analysis of β-catenin.* Lane 1, *normal gastric mucosa;* lane 2, *gastric cancer classified as β-catenin preserved type (+);* lane 3, *gastric cancer classified as β-catenin reduced type (±). The 95-kd band, full size β-catenin, was detected in all cancers that we examined in this study.* B: *Immunoblot analysis of β-catenin after immunoprecipitation using anti-E-cadherin antibody (HECD-1).* Lanes 1 *and* 2, *immunoprecipitated by normal mouse IgG1 as negative control, normal gastric mucosa and gastric cancer, respectively;* lane 3, *normal gastric mucosa;* lanes 4 *and* 5, *gastric cancers evaluated as β-catenin (+) and β-catenin (±), respectively.* Arrows *indicate full sized β-catenin (95-kd) molecules. The lower molecular mass bands are considered to be derived from immunoglobulin.*

7 (47%) E-cad±/β-cat±. Of the 19 gastric cancers examined, they were divided to 10 (53%) E-cad+/β-cat+, 5 (26%) E-cad+/β-cat±, and 4 (21%) E-cad±/β-cat±. In 22 colorectal cancers examined, 11 (50%), 5 (23%), and 6 (27%) were evaluated as E-cad+/β-cat+, E-cad+/β-cat±, and E-cad±/β-cat±, respectively. No statistically significant difference was observed between coexpression pattern and clinical features (data not shown). Figure 3 shows the staining pattern of an E-cad+/β-cat± tumor of gastric cancer.

Table 1. *Relationship between β-catenin Expression and Histological Grade*

Histological grade	β-Catenin expression	
	Preserved (+)	Reduced (±)
Esophageal cancer (n = 15)		
Well	4	4
Poor	1	6
Gastric cancer (n = 19)		
Well	3	2
Poor	7	7
Colorectal cancer (n = 22)*		
Well	9	3
Poor	2	8

*Fisher's exact test $P < 0.05$.

Table 2. *Coexpression of E-Cadherin and β-Catenin*

	E-cadherin/β-catenin		
	(+/+)	(+/±)	(±/±)
Esophageal cancer (n = 15)	5	3	7
Gastric cancer (n = 19)	10	5	4
Colorectal cancer (n = 22)	11	5	6

Discussion

In the present study, the noncancerous epithelium expressed β-catenin on cell-cell boundaries without exception, whereas 54% (30/56) of the cancers had reduced β-catenin expression. Considering the stable expression of β-catenin in noncancerous tissues, the reduction of the immunoreactivities of the cancer cells might be acquired through malignant transformation. Thus, the impaired expression of β-catenin is considered to be one of the characteristics for carcinoma. In the cancerous lesion, the location of immunoreactivities for β-catenin were predominantly associated with the cell membrane, especially at the cell-cell boundaries. In some tumors, diffuse and obscure immunostaining for β-catenin was observed in the cytoplasm. The distribution of immunoreactive β-catenin staining in cancer cells was similar to that for E-cadherin and α-catenin as described before.[5,26] These findings suggest that functional or structual disorders may occur in β-catenin molecules of some tumor cells along with reduced β-catenin expression.

In comparison with α- and β-catenin expression, α-catenin was down-regulated in 75.6% of the cancers including esophagus, stomach, and colorectal cancers in our previous studies.[26] Especially, α-catenin expression was completely negative in 70.6% of them.[26] However, in this study we found no cases with completely negative β-catenin expression or truncated β-catenin molecule using immunoblot analysis (data not shown). Through these findings, we suggest that the regulation of α-catenin and β-catenin expression may be controlled by different mechanisms.

Recent studies reported that β-catenin interacts more directly with the cytoplasmic domain of E-cadherin than α-catenin, which mediates the interaction

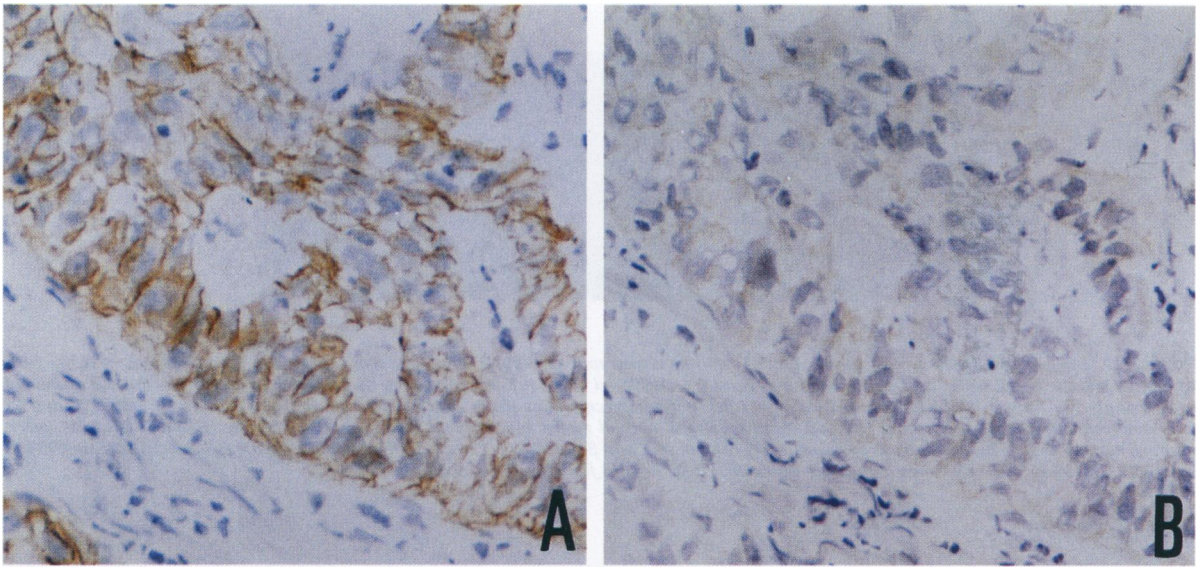

Figure 3. *Coexpression of E-cadherin and β-catenin in primary tumor of gastric cancer.* A: *Immunoreactive E-cadherin expression.* B: *Immunoreactive β-catenin expression. All cancerous cells express E-cadherin preserved but β-catenin reduced (classified as E-cad+/β-cat±). Original magnification, ×100.*

with the actin filament network,[27-29] and the mutation in β-catenin disrupts the interaction between E-cadherin and α-catenin.[30,31] From our results in the immunoprecipitation analysis, we also showed that β-catenin forms a complex with E-cadherin in human cancerous tissues as well as in normal epithelium *in vivo*. These results suggest that immunoreactive β-catenin have functional activities at least in the binding among E-cadherin, α-catenin, and cytoskeleton.

We proposed three mechanisms of alteration of the cadherin-mediated cell adhesion system in human cancers *in vivo* and *in vitro*. The first is down-regulation of E-cadherin expression and its gene mutation.[32,33] We have previously reported the existence of impaired E-cadherin expression in human cancers and a significant relationship between reduced E-cadherin expression and clinicopathological factors, such as dedifferentiation, invasiveness, or metastasis.[4-6] The second is abnormality or deletion of catenins.[10-12] As the function of cadherin is modulated by α-catenin, conceivable reduction of α-catenin expression could suppress cadherin-mediated cell-cell adhesion activity.[34] The third abnormality of this adhesion system is biochemical modification of catenins. We and others have recently demonstrated that tyrosine phosphorylation of β-catenin induced by various factors (eg, v-*src*, hepatocyte growth factor, and epidermal growth factor) suppresses E-cadherin function *in vitro*.[15,17,18] As tyrosine phosphorylation of β-catenin works as a signal transduction pathway, β-catenin might have the effect of down-regulating for E-cadherin function. β-Catenin might modulate cadherin-mediated cell adhesion activity during processes that require reduced intercellular adhesiveness, such as cell movement, cell migration, infiltration, and metastasis. From this biochemical background, we speculate that tyrosine phosphorylation of catenins is an important problem that should be further investigated.

In conclusion, we have shown various expression patterns of β-catenin in human cancerous tissues and the association between E-cadherin and β-catenin. As β-catenin forms a complex with E-cadherin in human epithelium and cancerous tissues, down-regulation of β-catenin expression may affect the E-cadherin-mediated adhesion system. More detailed analyses with a sufficient number of patients or coexpression of E-cadherin and catenins are necessary to clarify the cadherin-mediated cell adhesion system and the relationship with invasiveness and metastasis of cancer cells.

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