

Expression of adhesion molecules and mucins in human and rhesus macaque gastrointestinal epithelial cells

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Summary. Epithelial junctions and mucins play key roles in the gastrointestinal mucosal barrier, and their alterations are associated with numerous diseases, including carcinomas. The systematic expression of adhesion molecules and mucins in normal and malignant human gastrointestinal cells was investigated in this study. In normal human gastrointestinal cells, zonula occludens-1 (ZO-1), α -catenin, β -catenin, γ -catenin and desmoglein-2 (DSG2) were located in the cytoplasmic membranes, whereas symplekin stained in the nuclei. ZO-1, the three catenins, and DSG2 were observed in the gastric and colorectal carcinomas with reduced and heterogeneous expression and with abnormal distribution. Symplekin was detected in the nuclei of tumor cells in most tumors but not observed in some others. The immunohistochemical results for ZO-1 and symplekin on the tissues were consistent with the data for the cultured cells obtained by immunocytochemical staining and Western blot analysis. MUC1 was not stained in the normal gastrointestinal cells without periodate oxidation, but it was strongly labeled in the malignant gastrointestinal cells. MUC2 was detected in the normal and malignant gastrointestinal cells without the periodate treatment. These findings indicate that alterations in the expression of the epithelial junctions and mucins are associated with the malignant transformation of gastrointestinal cells. In addition, the gastrointestinal epithelial cells of rhesus macaques expressed these adhesion molecules and mucins, as did the human cells, suggesting that the rhesus monkey is a suitable experimental animal model for research on adhesion molecules and mucins.

Key words: Adhesion molecule, Mucin, Gastrointestinal epithelium, Carcinoma, Rhesus macaque

Introduction

The gastrointestinal mucosa acts as a barrier to maintain the stability of the internal environment of the alimentary tract and protects the tract from damage due to the degradative enzymes and such noxious substances as bacteria and toxins. This mucosal barrier is predominantly composed of epithelial junctions and mucins, alterations in which are associated with numerous diseases, including gastrointestinal tumors.

Epithelial junctions include tight junctions, adherens junctions and desmosomes. Transmembrane proteins (e.g., occludins, claudins, and JAMs) and cytoplasmic plaque proteins [e.g., symplekin, zonula occludens (ZO)-1, ZO-2, ZO-3, ZO-1-associated nucleic acid-binding protein (ZONAB), and cingulin] comprise the tight junctions. These proteins interact to form the core structure of the tight junctions (Shin et al., 2006). Adherens junctions are multiprotein complexes (E-cadherin/catenin complex) that mediate cell adhesion. E-cadherin and its associated cytoplasmic proteins, such as α -catenin, β -catenin, and γ -catenin, form the key functional component of adherens junctions between epithelial cells. Desmosomes are membranous specializations that anchor intermediate filaments to the plasma membrane and link cells together. Desmogleins (DSGs) are transmembranous glycoproteins of the desmosome that have three types of epidermal isoforms: DSG2 is expressed in simple epithelia, whereas DSG1 and DSG3 are restricted mostly to stratified squamous ones (Schäfer et al., 1994).

Mucins are a family of highly glycosylated glycoproteins present on the surface of many glandular

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epithelial cells and in their secretions. Their best known function is to provide a barrier between the luminal membranes of epithelial cells and their environment. They are classified into membrane-associated (MUC1) and membrane-secreted (MUC2) types, each having distinct characteristic protein domains and tissue-specific glycosylation.

Epithelial junctions and mucins exhibited alterations in malignant epithelial cells in our previous research (Cao et al., 1997a, 2007). In light of the association of epithelial junctions and mucins with malignant tumors, we further investigated the systematic expressions of tight junction proteins (symplekin and ZO-1), adherens junction proteins (α -, β -, and γ -catenins), DSG2, and epithelial mucins (MUC1 and MUC2) in normal and malignant tissues of human gastric and colon tissues using an *in situ* immunohistochemical approach. The objective of this study was to determine the expression and distribution of these important functional molecules in epithelial cells.

The rhesus macaque has been successfully used in studies on the pathological changes in gastrointestinal mucosae during human immunodeficiency virus or *Helicobacter pylori* infection (Mattapallil et al., 2000; Brenchley et al., 2004). Adhesion molecules and mucins have been previously investigated in humans, but the data on non-human primates are very limited. Thus, the present study also examined the molecules in the gastric and colon tissues of rhesus monkeys to obtain preliminary data for further experiments using this animal model.

Materials and methods

Tissue samples

The human samples included 3 normal stomach specimens, 3 normal colon specimens, 11 gastric carcinoma specimens, and 10 colorectal carcinoma specimens. These samples were surgically excised from 27 patients. The tumors were histologically classified according to Lauren (1965) and the recommendations of the World Health Organization (Hamilton and Aaltonen, 2000). The human samples were fully encoded to protect patient confidentiality. The present study was approved by the local research ethics committees at all participating sites. Three specimens from the normal stomach and colon of rhesus monkeys were also used. All tissue samples were fixed in 4% formalin for 24–48 h and embedded in paraffin.

Cell culture

Human cancer cell lines derived from gastric adenocarcinoma (SGC-7901 and HSC) and colorectal adenocarcinoma (Caco-2, SW480, and HT-29) were used in this study. The cells were cultured in Dulbecco's minimum essential medium (Gibco, Eggenstein, Germany) with 10% fetal bovine serum, 1% non-

essential amino acids, sodium pyruvate, and penicillin–streptomycin.

Antibodies

Anti-symplekin (clone 25) and anti-ZO-1 (clone 1) antibodies were purchased from BD Transduction Laboratories (San Jose, USA). Anti- α -catenin (clone 5), anti- β -catenin (clone 14), anti- γ -catenin (clone 15) and anti-DSG2 (clone 10G11-5-9) antibodies were obtained from Transduction Laboratories (Lexington, USA). MUC1 (clone A76-A/C7; Cao et al., 1997b) and MUC2 (clone 4F1; Devine et al., 1993) were kindly donated by Dr. U. Karsten (Berlin, Germany) and Dr. P. X. Xing (Heidelberg, Victoria, Australia), respectively.

Immunocytochemistry

The cells for immunofluorescence staining were seeded on glass cover slips in six-well plates. When the cells formed stable monolayers, they were air-dried and fixed with ice-cold acetone for 20 min. The cover slips were then air-dried and stored at -80°C until use.

The cells were fixed with ice-cold acetone for 10 min and rinsed with phosphate-buffered saline (PBS) before the immunostaining. After permeation with digitonin for 10 min, the cells were blocked with 2% bovine serum albumin (BSA) for 10 min. They were sequentially incubated for 1 hour at room temperature (RT) in humid chambers, with the primary antibodies diluted at 1:100 (symplekin, ZO-1, the three catenins, and DSG2). The cover slips were rinsed and incubated with Cy3-conjugated goat anti-mouse immunoglobulin (Ig) (Jackson ImmunoResearch Laboratories Inc., West Grove, USA) diluted at 1:100 away from light for 30 min at RT. They were then washed and stained with 4',6-diamidino-2-phenylindole diluted at 1:5000 for 5 min in a dark room. The cover slips were washed and mounted. Afterwards, the stained cells were visualized and captured by a fluorescent light microscope (Leica, Wetzlar, Germany).

Immunocytochemical staining was scored as follows: “-” for completely negative reactivity; “+” for partially and faintly positive reactivity; “++” for partially and distinctly positive reactivity; and “+++” for completely positive reactivity.

Immunohistochemistry

The paraffin sections were deparaffinized in xylene and hydrated through a graded series of ethanol. Antigen retrieval was performed using 0.01M sodium citrate buffer through pressure cooker processing for 5 min to detect symplekin, ZO-1, the three catenins, and DSG2. The sections were then rinsed with PBS. Endogenous peroxidase was blocked with 3% H_2O_2 for 5 min. After another round of rinsing with PBS, the sections were blocked with 2% BSA for 10 min and then incubated in humid chambers with primary antibodies for 1 h at RT.

The sections were then rinsed and incubated for 30 min at RT with peroxidase-conjugated goat anti-mouse Ig. 3,3'-diaminobenzidine was used as the peroxidase substrate. The slides were counterstained with hematoxylin. The primary antibodies were replaced with a comparable dilution of mouse IgG as negative control.

Periodate oxidation was performed on sections of rhesus macaque samples before they were incubated with anti-MUC1 mAb to determine whether MUC1 exists in the gastrointestinal epithelial cells of these animals. The sections were incubated for 30 min at RT with 20 mM periodic acid in acetate buffer (0.05 M, pH 5). After three rinses with PBS, they were treated with 1% glycine for 30 min and further rinsed three times with PBS. This pretreatment eliminated the glycotopes of glycoproteins without damaging the peptide epitopes and was used for detecting antigens masked by glycans (Cao et al., 1998).

Scoring of the immunohistochemical staining was based on membranous reactivity. The intensities and patterns of the membranous staining were evaluated using the following scale (Hofmann et al., 2008): “-” for completely negative or membranous reactivity in <10% of cells; “+” for faint and partial membranous reactivity in >10% of cells; “++” for moderate and complete membranous reactivity in >10% of cells; and “+++” for strong and complete membranous reactivity in >10% of cells. Positive nuclear or cytoplasmic reactivity was directly labeled as “U” or “C”, respectively. The scoring was confirmed by a second independent examination.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis

After confluence, the stock culture cells were dissociated using 0.05% trypsin and 0.53 mM EDTA, collected, placed in a radioimmunoprecipitation assay buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1.5 mM EDTA, and 1 mM NaVanadate) containing phenyl-methanesulfonyl fluoride at a final concentration of 5 mmol/L and Protease Inhibitor Cocktail Set III (Merck KGaA, Darmstadt, Germany) with 1:100 dilution, and then thoroughly mixed on ice for 30 min using a vortexer. The lysate was centrifuged at 12,000g for 5 min at 4°C, and the supernatants were collected and stored frozen (-20°C) until use. The total protein concentration was determined using bicinchoninic acid protein assay (P0012, Beyotime, Shanghai, China). The protein extracts were boiled in SDS-PAGE reducing buffer for 10 min, separated by SDS-PAGE, and then transferred to a polyvinylidene difluoride transfer membrane. Twenty micrograms of protein was applied per lane on the mini gels. The membrane was blocked with 1% BSA and 0.2% Tween-20 in Tris-buffered saline (TBS) for 2 h, and then incubated with the first antibodies (anti-symplekin and anti-ZO-1) for 1 h at RT. After washing with 0.2% Tween-20 in TBS, the membrane was treated with peroxidase-conjugated secondary antibody (No. PO447, DAKO, Copenhagen,

Denmark) for 1 h at RT. Finally, the membrane was incubated with SuperSignal West Pico chemiluminescent substrate (Thermo Fisher Scientific Inc., Waltham, MA, USA), and the blots were exposed to X-ray film from 1 to 10 min. The films were developed, scanned, and analyzed using Image J (NIH, Bethesda, USA). β -Actin was used as an internal control.

Results

Expression of the adhesion molecules in the culture cells

Symplekin was stained in the nuclei of the cultured cells (SGC-7901, HSC, Caco-2, SW480, and HT-29), but it was not present at the membrane and in the cytoplasm (Fig. 1A). ZO-1 displayed intensive staining at the site of cell-cell contacts of Caco-2 cells (Fig. 1B), membranous and cytoplasmic staining in SW480 and HT-29 cells, weak membranous staining in HSC cells, negative membranous staining in SGC-7901 cells, and faint nuclear staining in HT-29 cells. Catenins showed generally weak/negative membranous staining and aberrant cytoplasmic distribution in all the cell lines used. The HSC, SW480, and HT-29 cell membranes did not express α -catenin. Membranous and cytoplasmic distribution of β -catenin was detected in the SGC-7901, Caco-2 (Fig. 1C), SW480, and HT-29 cells. Similarly, γ -catenin was present at the membrane and in the cytoplasm of SGC-7901 (Fig. 1D), Caco-2, and HT-29 cells but absent at the HSC and SW480 cell membranes. The results of immunofluorescence staining are listed in Table 1.

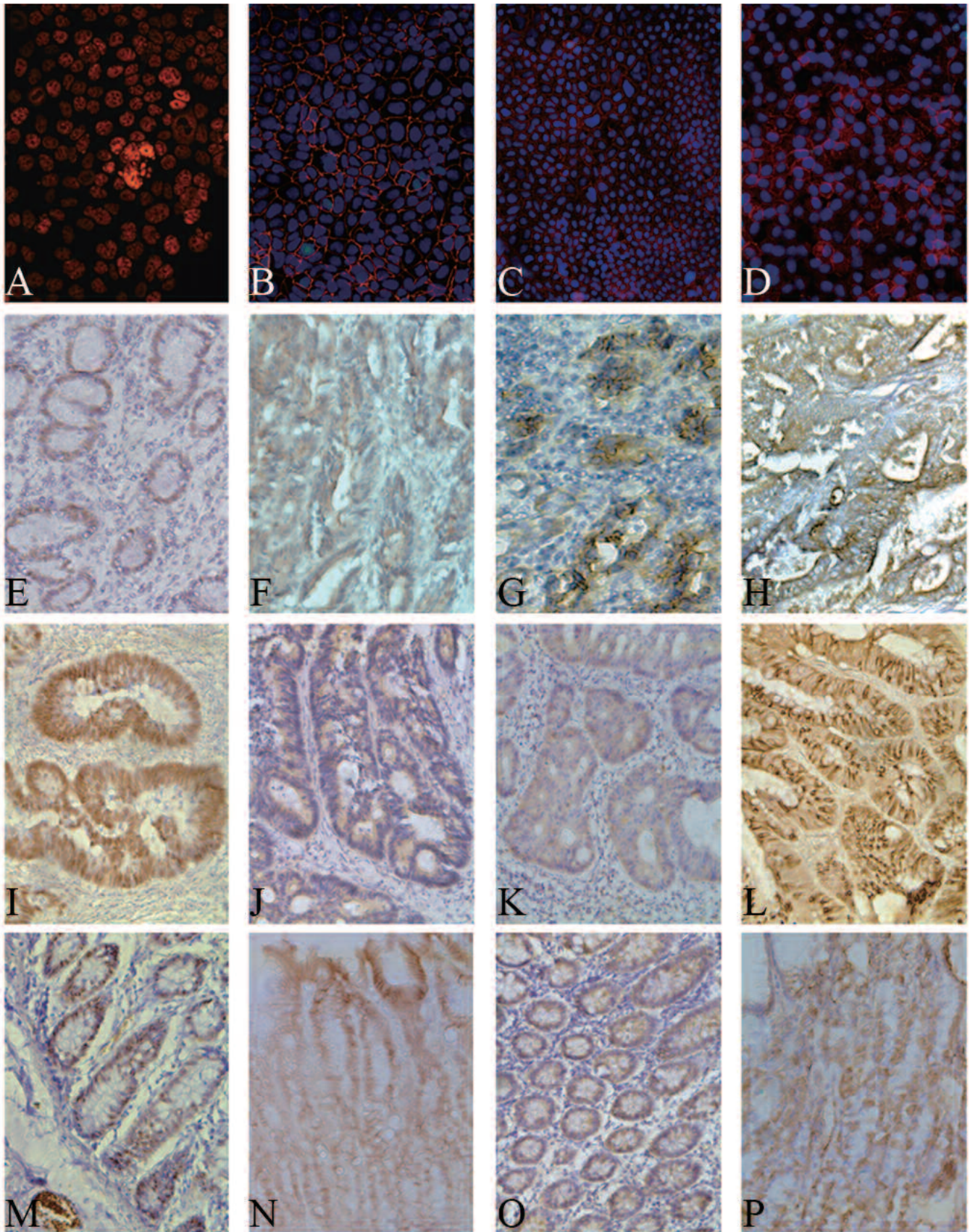
Western blot analysis was performed to examine the expressions of symplekin (126.5 kDa) and ZO-1 (220 kDa) in SGC-7901, HSC, Caco-2, SW480, and HT-29. Symplekin was detected in all cell lines (Fig. 2), and its expression values varied accordingly. SGC-7901 showed weak expression of symplekin compared with HT-29, Caco-2, and HSC, but SW480 exhibited the lowest level of expression. ZO-1, also detected in all cell lines (Fig.

Table 1. Expression of the adhesion molecules in the cell lines.

| Adhesion molecules | SGC-7901 | HSC | Caco-2 | SW480 | HT-29 |
|----------------------------|----------|------|---------|--------|----------|
| Tight junctions | | | | | |
| Symplekin | U++ | U++ | U++ | U+ | U+++ |
| ZO-1 | C+ | M+ | M+++ | M++/C+ | M+/C+/U+ |
| Adhesions junctions | | | | | |
| α -Catenin | M+/C+ | C+ | M+/C+ | C+ | C+ |
| β -Catenin | M+/C++ | C++ | M+++/C+ | M+/C++ | M+/C+ |
| γ -Catenin | M++/C+ | C+ | M+++/C+ | C+ | M++/C+ |
| Desmosome | | | | | |
| DSG2 | M+/C++ | C+++ | M+/C++ | C++ | M+/C++ |

M: present in the plasma membrane; C: present in the cytoplasm; U: present in the nucleus. Scoring: “-”, completely negative; “+”, partially and faintly positive; “++”, partially and distinctly positive; and “+++”, completely positive.

Junctions and mucins in gastric and colon tissues



Junctions and mucins in gastric and colon tissues

Fig. 1. A-D. Immunofluorescence staining of symplekin, ZO-1, β -catenin, and γ -catenin in the culture cells. **A.** Symplekin was stained in the nuclei of Caco-2 cells. **B.** Staining of ZO-1 was observed at the site of cell-cell contacts of Caco-2 cells. **C.** β -Catenin showed membranous and cytoplasmic distribution in Caco-2 cells. **D.** γ -Catenin displayed membranous and cytoplasmic expression in SGC-7901 cells. **E-H.** Immunohistochemical analysis of symplekin, ZO-1, γ -catenin, and MUC1 in the gastric carcinomas. **E.** Symplekin was detected in the nuclei of tumor cells. **F.** ZO-1 showed membranous and cytoplasmic expression in the tumor cells. **G.** Expression of γ -catenin diminished at the membrane of tumor cells. **H.** MUC1 showed intensive distribution at the membrane and in the cytoplasm of tumor cells. **I-L.** Immunohistochemical analysis of symplekin, ZO-1, DSG2, and MUC2 in the colorectal carcinomas. **I.** Symplekin was stained in the nuclei of tumor cells. **J.** Reduced expression of ZO-1 was observed at the membrane of tumor cells. **K.** DSG2 displayed cytoplasmic accumulation in the tumor cells. **L.** MUC2 showed membranous and cytoplasmic overexpression in the tumor cells. **M-P.** Immunohistochemical analysis of symplekin, α -catenin, β -catenin and MUC2 in the normal stomach and colon of rhesus macaques. **M.** Colon: Symplekin was stained in the nuclei of epithelial cells. **N.** Stomach: α -Catenin was located in the membrane of epithelial cells. **O.** Colon: β -Catenin showed membranous expression in the glandular epithelial cells. **P.** Stomach: MUC2 displayed membranous and cytoplasmic localization in the epithelial cells. A-F, H-P, x 200; G, x 400.

3), was strongly expressed in Caco-2 and SW480, moderately expressed in HT-29 and HSC, and weakly expressed in SGC-7901. The expression values of both proteins determined by Western blot analysis were consistent with the immunocytochemical results.

Expression of the adhesion molecules and mucins in the normal human stomach and colon

Symplekin was stained in the nuclei of the epithelial cells and lymphocytes. ZO-1, α -catenin, β -catenin, γ -catenin, and DSG2 were homogeneously expressed at the superficial and glandular epithelial cell membranes.

MUC1 was not labeled in the normal human stomach and colon, whereas MUC2 stained at the membrane and in the cytoplasm of the epithelial cells. The immunohistological results are shown in Table 2.

Expression of the adhesion molecules and mucins in human gastric and colorectal carcinomas

The tumor cells did not express symplekin in 5 (45%) of 11 gastric adenocarcinomas. Symplekin was stained in the nuclei of the positive cases (Fig. 1E). Membranous or cytoplasmic staining of symplekin was not observed. However, ZO-1 displayed membranous and cytoplasmic staining (Fig. 1F), cytoplasmic accumulation, and negative staining in 8 (73%), 1 (9%), and 2 (18%) of the 11 gastric adenocarcinomas,

respectively. Instead of regular expression in the normal human stomach epithelial cells, the catenins and DSG2 were heterogeneously expressed at the plasmic membrane and in the diffuse cytoplasm of the tumor cells. α -Catenin exhibited negative staining in 9 (82%) of 11 tumor cases, and weak membranous and

Table 2. Expression of the adhesion molecules and mucins in the normal gastrointestinal epithelial cells of humans and rhesus macaques.

| Adhesion molecules and mucins | Humans | | rhesus monkeys | |
|-------------------------------|---------|-------|----------------|-------|
| | Stomach | Colon | Stomach | Colon |
| Tight junctions | | | | |
| Symplekin | U | U | U | U |
| ZO-1 | M++ | M++ | M++ | M++ |
| Adherens junctions | | | | |
| α -Catenin | M++ | M++ | M++ | M++ |
| β -Catenin | M++ | M++ | M++ | M++ |
| γ -Catenin | M++ | M++ | M++ | M++ |
| Desmosome | | | | |
| DSG2 | M++ | M++ | M++ | M++ |
| Mucins | | | | |
| MUC1 | - | - | M++* | M++* |
| MUC2 | M++/C | M++/C | M++/C | M++/C |

See Materials and methods for details on scoring. *MUC1 was expressed in the gastrointestinal epithelial cells of rhesus monkeys after treatment with periodate.

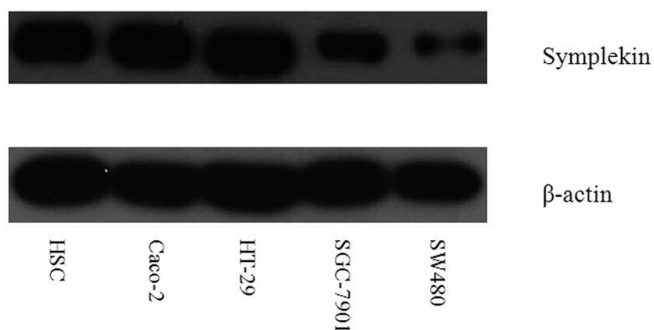


Fig. 2. Results of Western blot analysis of symplekin in the cell lines.

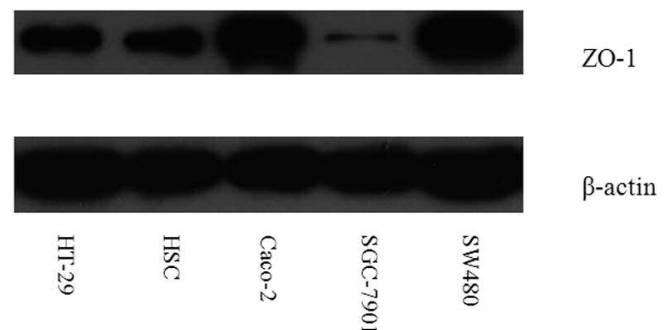


Fig. 3. Results of Western blot analysis of ZO-1 in the cell lines.

Table 3. Expression of the adhesion molecules and mucins in gastric and colorectal carcinomas.

| Adhesion molecules and mucins | Gastric carcinomas (11 cases) | Colorectal carcinomas (10 cases) |
|-------------------------------|-------------------------------|----------------------------------|
| Tight junctions | | |
| Symplekin | 5U | 8U |
| ZO-1 | 5M++; 3M+/C; 1C | 7M++; 2M+/C |
| Adherens junctions | | |
| α -Catenin | 2M+/C | 3M+/C |
| β -Catenin | 8M++; 3M+/C | 7M++; 3M+/C |
| γ -Catenin | 7M++; 4M+/C | 5M++; 2M+ |
| Desmosome | | |
| DSG2 | 2C | 4C |
| Mucins | | |
| MUC1 | 11M+++/C | 10M+++/C |
| MUC2 | 11M+++/C | 10M+++/C |

Values indicate the number of positive cases. See Materials and methods for details on scoring.

cytoplasmic staining in the other cases. β -Catenin displayed membranous and aberrant cytoplasmic distribution in the tumor cells. The expression of γ -catenin (Fig. 1G) was similar to that of β -catenin. DSG2 was absent in 9 cases (82%) but present in the cytoplasm in 2 (18%) of the 11 tumor cases.

The expression of the adhesion molecules in the colorectal adenocarcinomas resembled the results for the gastric carcinomas. Symplekin was absent in 2 cases (20%) of colorectal carcinoma, but it was detected in the nuclei of the tumor cells in 8 of 10 cases (Fig. 1I). ZO-1 was positive in 9 cases (90%) (Fig. 1J). α -Catenin displayed negative expression in 7 cases (70%) and abnormal staining in 3 (30%). β -Catenin showed abnormal cytoplasmic localization in 3 cases (30%). The expression of γ -catenin was similar to that of β -catenin in most cases, except for the 3 negative cases. DSG2 was absent in 6 tumor cases (60%) but present in the diffuse cytoplasm of the tumor cells in 4 cases (40%) (Fig. 1K).

MUC1 revealed intensive membranous and cytoplasmic localization in the gastric and colorectal adenocarcinomas (Fig. 1H), whereas MUC2 was excessively expressed at the membrane and in the cytoplasm of the tumor cells in all tumors (Fig. 1L). The results of immunohistological staining are summarized in Table 3.

Expression of the adhesion molecules and mucins in normal gastric and colon tissues of rhesus macaques

Symplekin was stained in the nuclei of rhesus macaque gastrointestinal epithelial cells and lymphocytes (Fig. 1M). ZO-1, α -catenin (Fig. 1N), β -catenin (Fig. 1O), γ -catenin, and DSG2 were expressed at the membrane of the superficial and glandular epithelial cells. After periodate oxidation, MUC1 was detected in the epithelial cell membrane. MUC2 was

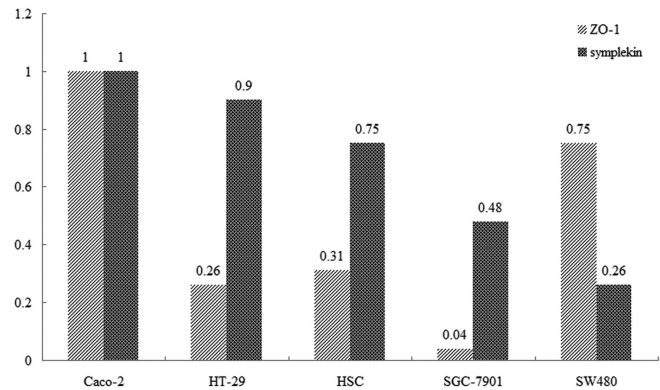


Fig. 4. Comparison of ZO-1 expression with symplekin expression in the cell lines using Western blot analysis.

located in the membrane and in the epithelial cell cytoplasm (Fig. 1P). The immunohistological results are shown in Table 2.

Discussion

The epithelial junction is essential for maintaining the integrity of the gastrointestinal epithelium, which is in turn necessary for the mucosal barrier. Tight junctions, adherens junctions, and desmosomes are the major components of these junctions.

Tight junctions play a critical role in paracellular permeability and cellular polarity. Data on the expression of the tight junction proteins in gastrointestinal epithelial cells are very limited. ZO-1, an important tight junction protein, is a peripheral membrane phosphoprotein and is located in the apical membrane of the normal epithelial cell. As a tight junction scaffolding or adaptor protein, ZO-1 forms complexes with ZO-2, ZO-3, and other proteins. In addition, ZO-1 belongs to the family of membrane-associated guanylate kinase proteins, regulates intracellular signaling and cell proliferation (Anderson, 1996), and suppresses tumors via its Src homology-3 and PDZ domains (Balda et al., 1996; Martin and Jiang, 2009). Previous research has shown that the expression of ZO-1 was reduced in gastric carcinomas (Ohtani et al., 2009) and that the expression levels of ZO-1 were markedly decreased in colorectal tumors with liver metastasis (Kaihara et al., 2003). Downregulation of ZO-1 and its failure to accumulate at cell junctions may be causally related to cancer progression (Hoover et al., 1998). In the present study, ZO-1 was regularly distributed at the apical membrane of normal human gastrointestinal epithelial cells but absent or abnormally localized in malignant gastrointestinal epithelial cells. ZO-1 was markedly detected in some nuclei of the tumor cells, indicating that it targets the nucleus of the subconfluent epithelial cell before the tight junctions mature. The nuclear distribution of ZO-1 is inversely

related to the extent and/or maturity of cell contact (Gottardi et al., 1996), and ZO-1 exhibits nuclear localization and nuclear export signals that enable it to shuttle between the cytoplasm and the nucleus (Bauer et al., 2010). These data suggest that the ectopic expression and downregulation of ZO-1 could play a role in the tumorigenesis of the gastrointestinal epithelium.

Symplekin is a dual-localization tight junction protein that is enriched in the nucleus and associates with tight junctions in polarized epithelial cells (Keon et al., 1996). It is involved in cytoplasmic RNA polyadenylation and transcriptional regulation (Xing et al., 2004; Kolev and Steitz, 2005). The nuclear symplekin cooperates with the Y-box transcription factor ZONAB to increase the transcription of cell cycle-related genes and inhibits differentiation of intestinal cells (Balda et al., 2003). Symplekin negatively regulates intestinal goblet cell differentiation in coordination with ZONAB (Buchert et al., 2009, 2010). The anti-symplekin antibody (BD Transduction Laboratory) was used in the present study to label symplekin in normal and malignant human gastrointestinal epithelial tissues. Only nuclear staining was observed, similar to previous research (Buchert et al., 2010). Some cases of gastric and colorectal carcinomas were negative for symplekin. The localizations of symplekin on the gastric and colorectal carcinoma tissues were confirmed on the cultured cancer cells by immunocytochemistry. In our previous study (Cao et al., 2007), most cancer cells lost the ability to express symplekin in poorly differentiated hepatocellular carcinomas. However, upregulated symplekin in the nucleus may promote tumorigenesis in the human colorectal epithelium (Buchert et al., 2010). The pathophysiological roles and significance of abnormal expression of symplekin in gastrointestinal carcinomas hence require further investigation.

Comparison of the expression values of ZO-1 and symplekin in the same culture cells (Fig. 4) revealed a correlative trend in most cell lines: the tumor cells that tested intensely positive for ZO-1 also strongly expressed symplekin, and vice versa. However, an inverse correlation between the two proteins in the SW480 cells, which displayed intensive expression of ZO-1 but weak expression of symplekin, was also observed. In the tight junction, ZO-1 acts as an adaptor or scaffolding protein binding other tight junction proteins. Both ZO-1 and symplekin are located in the plaque of tight junctions. However, further research on the ambiguous relationship between ZO-1 and symplekin is needed.

Adherens junctions are complex structures and contain the transmembrane glycoprotein (E-cadherin) and three submembranous cytosolic proteins (α -, β -, and γ -catenins). β - or γ -Catenin (plakoglobin) directly binds to the cytoplasmic tail of E-cadherin, whereas α -catenin links the bound β - or γ -catenin to the actin cytoskeleton. Numerous immunohistochemical studies on human cancers have shown that a proportion of invasive carcinomas and carcinomas in situ, including gastric and

colorectal cancers, aberrantly expressed E-cadherin or catenins compared with the related normal tissues (El-Bahrawy et al., 2001; Joo et al., 2001). In the present study, the catenins were located in the lateral membrane of the normal gastrointestinal epithelial cells, as previously described (Valizadeh et al., 1997; Yu et al., 2000). In contrast to the normal epithelial cells, cytoplasmic accumulation and reduced membranous staining of the three catenins were observed in the human gastric and colorectal carcinomas, as well as in the cultured cancer cells, which agrees well with previous research (Valizadeh et al., 1997; Yu et al., 2000; Joo et al., 2001). Considered as the key component of the Wnt/Wg signaling pathway, the abnormal expression of β -catenin in gastric and colorectal carcinomas is apparently important (Ebert et al., 2003; Lee et al., 2009; Pancione et al., 2010). β -Catenin is located in the nuclei of tumor cells when there is nuclear translocation or β -catenin gene mutation (Kobayashi et al., 2000; Clements et al., 2002). Abnormal expression and distribution of the E-cadherin/catenin complex may reduce the adherence between epithelial cells, interfere with cellular signaling, and induce loss of cell polarity — all these are involved in the development and progression of gastrointestinal cancer.

Desmosomes are punctate membrane domains traversed by representatives of two types of desmosomal cadherins, namely, DSGs and desmocollins. DSG2 was expressed at the lateral membrane of the normal gastrointestinal epithelial cells, as previously described (Schäfer et al., 1994). In previous research, we found that the expression of DSG2 was reduced in hepatocellular carcinomas (Cao et al., 2007); in the present study, decreased expression and aberrant distribution of DSG2 were observed in the gastric and colorectal carcinoma tissues and cultured cells, similar to other studies (Biedermann et al., 2005; Yashiro et al., 2006). The association of DSGs with tumor development (Tselepis et al., 1998) suggests that the abnormal expression of DSG2 may contribute to the progression of gastrointestinal cancer. This topic will be addressed in further studies.

Mucins, which are hydrolyzed by proteases with difficulty, are an essential component of the mucosal barrier. These consist of a mucin core protein backbone (apomucin) and numerous O-linked oligosaccharides (Gendler and Spicer, 1995). Abnormal expression of mucins has been viewed as one of the most prominent characteristics of numerous types of cancers and inflammatory diseases (Hollingsworth and Swanson, 2004; Moniaux et al., 2004). In the normal gastrointestinal epithelium the immunohistochemical staining of MUC1 is hindered by tissue-specific glycosylation, which protects the epithelial cells from the overwhelming presence of proteases in the gastrointestinal tract (Cao et al., 1997a). As a prototypical member of the membrane-bound mucin subfamily, MUC1 has long been considered as a tumor-associated molecule because of its frequent

overexpression and aberrant glycosylation in most carcinomas (Gendler, 2001; Hollingsworth and Swanson, 2004). In the present study, the normal human gastric and colon epithelial cells negatively reacted with the anti-MUC1 mAb (A76-A/C7). In contrast, MUC1 was stained at the membrane and in the cytoplasm of the gastric and colorectal carcinoma cells. The positive reaction and distribution of MUC1 in the tumors resulted from its alteration of glycosylation and cellular localization occurring in the malignant transformation. Accordingly, the over-expression and non-apical distribution of MUC1 may play an active part in the development and progression of gastrointestinal cancer (Cao et al., 1997b). MUC2 is characterized as an intestinal goblet cell mucin (Tytgat et al., 1994; Ajioka et al., 1997) and detected in normal and malignant colonic tissues. Ectopic expression of MUC2 is known to occur in the intestinal metaplasia of gastric mucosae (Correa, 1988). In the present study, unlike MUC1, MUC2 was positively stained in the normal gastrointestinal epithelial cells; in addition, MUC2 was over-expressed in the gastric and colorectal carcinomas. Consequently, overexpression of MUC2 may be correlated with the tumorigenesis of the gastrointestinal epithelium.

The expression of adhesion molecules (sympleskin, ZO-1, the three catenins, and DSG2) and mucins in normal rhesus macaque gastric and colorectal tissues was also investigated in this study. The normal rhesus macaque gastrointestinal epithelial cells expressed these adhesion molecules, as expected. MUC1 was positive in the normal rhesus macaque gastrointestinal epithelial cells after periodate treatment, whereas MUC2 was detected without it. The results for the tested animals were similar to those for the human subjects, making the rhesus monkey a suitable experimental animal model for research on the functions of the adhesion molecules and mucins, as well as on the relationships between these molecules and diseases. Further studies on this topic should hence be considered.

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