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# Expression of beta-catenin and its mechanism of delocalization in intestinal-type early gastric cancer based on mucin expression

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**Summary.** The biological characteristics of intestinal-type early gastric cancers (ICs) differ based on mucin phenotypes. Beta-catenin delocalization is a predictive marker of aggressive biological behavior (submucosal invasion and lymph node metastasis) of ICs. The presumptive causative genetic alterations leading to delocalization of beta-catenin in ICs are still controversial, and there are only a few reports regarding beta-catenin expression in gastric cancer based on mucin phenotypes. Therefore, in the current study, the expression and mechanisms of delocalization of beta-catenin were elucidated on the basis of mucin phenotypes in 109 cases of ICs. There was increased cytoplasmic and nuclear beta-catenin expression (delocalization) in ICs with a predominant intestinal mucin phenotype (ICIP; 46.3% [25/54 cases]) compared to ICs with a predominant gastric mucin phenotype (ICGP; 20% [11/55 cases]). There were no beta-catenin or APC mutations in ICs. APC promoter hypermethylation was present in 49 of 105 (46.7%) cases of ICs. There was a significant relationship between APC promoter hypermethylation and beta-catenin delocalization in ICs, especially in ICIPs. There was no relationship between beta-catenin delocalization and APC gene loss of heterozygosity in ICs. In conclusion, we showed that beta-catenin delocalization was more evident in ICIPs, and APC promoter hypermethylation might play a role in delocalization of beta-catenin, especially in ICIPs.

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**Key words:** Early gastric cancer, Beta-catenin, Mucin, Methylation

## Introduction

Gastric cancer remains one of the most prevalent malignancies worldwide. More than 90% of gastric cancers are adenocarcinomas, which are divided into two histologic types (differentiated and undifferentiated, or intestinal and diffuse) based on the tendency of gland formation (Lauren, 1965; Nakamura et al., 1968). The pathogenesis of intestinal-type gastric adenocarcinoma has been connected to precursor changes, such as chronic atrophic gastritis, intestinal metaplasia, and adenoma, whereas the diffuse type lacks well-recognized precursor lesions (Stadtlander and Waterbor, 1999; Schlemper et al., 2000). On the basis of mucin phenotypes, intestinal-type gastric carcinomas (ICs) show mostly intestinal-type mucin, but some ICs have been shown to have gastric-type mucin (Tatematsu et al., 1990; Saito et al., 2001). Furthermore, it has become clear that the biological characteristics of ICs differ based on mucin phenotypes (Koseki et al., 2000; Kushima et al., 2003). Thus, ICs with gastric mucin have increased malignant potential compared to those with intestinal-type mucin.

Beta-catenin is an important mediator of the Wnt signaling pathway, as well as cell-to-cell adhesion (Peifer, 1995, 1997; Resnik, 1997). The intracellular degradation of beta-catenin is regulated by the adenomatous polyposis coli (APC) protein and glycogen synthetase kinase (GSK)-3 beta (Rubinfeld et al., 1996). The activated Wnt signaling pathway inhibits degradation of beta-catenin and leads to the

accumulation of cytoplasmic beta-catenin and translocation of beta-catenin into the nucleus. Nuclear-beta catenin forms heterodimers with members of the T-cell factor (TCF) family of transcription factors and a beta-catenin/TCF-activated gene transcription can increase cellular proliferation (Polakis, 2000). Therefore, genetic alteration of APC, beta-catenin, and other components of the Wnt signaling pathway results in impaired degradation of beta-catenin and increased cellular and nuclear beta-catenin accumulation (delocalization), and can induce neoplastic transformation (Kinzler and Vogelstein, 1996; Bienz and Clevers, 2000; Satoh et al., 2000). There are a number of reports regarding beta-catenin alteration and expression in gastric cancer (Miyazawa et al., 2000; Sasaki et al., 2001; Grabsch et al., 2001; Park et al., 1999; Koppert et al., 2004; Aihara et al., 2005; Ogasawara et al., 2006). Miyazawa et al. (2000) showed nuclear expression of beta-catenin in 12% of gastric cancers, all of which were intestinal-type (differentiated) adenocarcinomas and suggested that nuclear accumulation of beta-catenin is associated with early tumor invasion in intestinal-type gastric carcinoma. However, the presumptive causative genetic alterations leading to delocalization of beta-catenin in ICs are still controversial and there are only a few reports about beta-catenin expression in gastric cancer based on mucin phenotypes in ICs.

Therefore, we tried to elucidate the presumptive mechanisms of delocalization of beta-catenin in gastric cancer on the basis on mucin phenotypes, especially in early intestinal gastric adenocarcinomas.

## Materials and methods

### *Clinicopathologic characteristics of patients*

One hundred and nine patients (85 males and 24 females) with a mean age of 59.0 years (range, 42-75 years) who were diagnosed with early gastric cancers and a differentiated (intestinal) phenotype based on the Lauren classification, and who underwent gastrectomy with lymph node dissection at Pusan National University Hospital between 2002 and 2003 were included in this study. Formalin-fixed and paraffin embedded specimens were kindly provided by the National Biobank of Korea of PNUH. No preoperative radiotherapy and/or chemotherapy had been administered. The clinicopathologic findings, such as age, gender, tumor site, tumor size, histologic type, depth of invasion, lymphovascular invasion, and lymph node metastasis were reviewed according to the Japanese Classification of Gastric Carcinomas (Japanese Gastric Cancer Association, 1998). This study was approved by the Institutional Review Board at Pusan National University Hospital after obtaining informed consent.

### *Immunohistochemistry for mucin phenotypes*

Sections were dewaxed and rehydrated according to standard procedures, and washed with PBS. For immunohistochemical staining, sections were heated in a microwave oven at 600W for 2x5 minutes in 0.01M citrate buffer (pH 6.0). Sections were immersed in 3% $H_20_2$ to quench endogenous peroxidase activity, and unspecified binding was blocked in 5% normal goat serum (0.1% BSA in PBS). Immunohistochemical staining was performed by the avidin-biotin peroxidase complex method with aminoethylcarbazole as a chromogen using the Vetastain ABC elite kit (Vector Laboratories, Burlingame, CA, USA), according to the manufacturer's instructions. Sections were counterstained with Mayer's hematoxylin solution. We used primary antibodies for MUC-5AC, MUC-6, MUC-2, and CD10 as shown in Table 1. MUC-5AC and MUC-6 are markers of the gastric phenotypes, whereas MUC-2 and CD10 are typical of the intestinal phenotypes. Gastric cancers with >10% of the positive area for each mucin were identified as positive phenotypes. Mucin

**Table 1.** Clinicopathologic characteristics and mucin phenotypes of intestinal-type early gastric cancers.

| | Case No. | Beta-catenin delocalization | | p value |
|---|---|---|---|---|
| | | Negative | Positive | |
| Age (years) | | | | |
| ≤ 50 | 19 | 14 | 5 | 0.494 |
| > 50 | 90 | 59 | 31 | |
| Gender | | | | |
| Male | 85 | 53 | 32 | 0.054 |
| Female | 24 | 20 | 4 | |
| Tumor size (cm) | | | | |
| ≤ 2 | 51 | 35 | 16 | 0.730 |
| > 2 | 58 | 38 | 20 | |
| Location | | | | |
| Upper | 33 | 23 | 10 | 0.690 |
| Middle and lower | 76 | 50 | 26 | |
| Invasion Depth | | | | |
| Mucosa | 53 | 39 | 14 | 0.153 |
| Submucosa | 56 | 34 | 22 | |
| Gross type | | | | |
| Elevated/flat | 49 | 38 | 11 | 0.034 |
| Depressed | 60 | 35 | 25 | |
| Lymphovascular emboli | | | | |
| Negative | 85 | 60 | 25 | 0.131 |
| Positive | 24 | 13 | 11 | |
| Lymph node metastasis | | | | |
| Negative | 94 | 65 | 29 | 0.247 |
| Positive | 15 | 8 | 7 | |
| Mucin Phenotypes* | | | | |
| ICGP (G type) | 32 | 25 | 7 | 0.004 |
| ICGP (GI[G] type) | 23 | 19 | 4 | |
| ICIP (GI[I] type) | 9 | 3 | 6 | |
| ICIP (I type) | 45 | 26 | 19 | |

*: ICGP (G plus GI[G] types) vs. ICIP (GI[I] plus I types). Note: ICGP; intestinal type early gastric cancer with gastric mucin predominant type ICIP; intestinal type early gastric cancer with intestinal mucin predominant type.

phenotypes were further subdivided into gastric (G; only gastric mucin-positive) and gastric predominant gastrointestinal phenotypes (GI[G]), intestinal (I; only intestinal mucin-positive), and intestinal predominant gastrointestinal phenotypes (GI[I]) based on a combination of predominant patterns of MUC5AC, MUC-2, MUC-6, and CD10 staining (Tsukashita et al., 2001). We defined intestinal-type early gastric cancer with predominant gastric mucin type (ICGP) as gastric-type plus gastric predominant gastrointestinal phenotypes and intestinal-type early gastric cancer with predominant intestinal mucin type (ICIP) as intestinal-type plus intestinal predominant gastrointestinal phenotypes.

### *Immunohistochemistry for beta-catenin*

Briefly, 4$\mu$m-thick consecutive sections were deparaffinized and hydrated through a graded series of alcohol. We used the same immunohistochemical method as described above and primary antibody for beta-catenin (1:100, mouse monoclonal antibody 14; Transduction Laboratories, Lexington, KY, USA). The immunostaining patterns (membranous, cytoplasmic, or nuclear) were analyzed. A case was recorded as positive if >10% of the epithelial cells showed immunoreactivity.

### *Beta-catenin mutation analysis*

Genomic DNA was extracted from the tumor area and microdissected from the formalin-fixed paraffin-embedded sections using a Takara DEXPAT™ kit (Takara Bio Inc.,Shiga, Japan). The tumor DNA was evaluated for mutation in the GSK-3 beta phosphorylation consensus motif of the beta-catenin gene using polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP). The DNA sequence of the third coding exon of the beta-catenin gene was amplified using the forward 5'-primer (GAT TTG ATG GAG TTG GAC ATG G) and the reverse 3'-primer (TGT TCT TGA GTG AAG GAC TGA G). PCR was carried out in a 25 $\mu$l reaction mixture containing 50 ng of genomic DNA, 2.5 $\mu$l of each primer, 4l of each dNTP, 10 $\mu$l PCR buffer, and 0.25U (1.25U) of Taq polymerase. The PCR reaction conditions were as follows: 5 min at 95$\mu$l and 40 cycles of denaturation at 94°C for 30 sec, annealing at 62°C for 30 sec, and extension at 72°C for 60 sec on a Gene Amp PCR System 9600. For SSCP analysis, 10°C of the PCR product was denatured by adding 10°C of stop solution (95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol, and 20mM EDTA) and heated at 95°C for 5 min. The samples were then loaded onto a 12% polyacrylamide gel and run for 4h at 12mA at 4°C. After electrophoresis, the DNA was visualized by silver staining using a Silver stain plus kit (Biorad, Hercules, CA, USA). The bands detected were cut out, and the PCR was repeated using the same primers. The re-amplified PCR products were sequenced using an automatic sequencer.

### *APC mutation analysis*

APC mutation analysis was performed using PCR-SSCP and direct sequencing in 35 of 108 cases presented nuclear expression in immunohistochemistry for beta-catenin. Overlapping primer sets numbered 1-7 (5' to 3') were designed for genomic DNA. Screening of APC involved exon 15 codons 1274-1523. The primer sequences are 1U 5'-(ACT CCA ATA TGT TTT CAA GAT G)-3' and 1D 5'-(GGA ACT TCG CTC ACA GGA T)-3'; 2U 5'-(GCA GAT TCT GCT AAT ACC CT)-3' and 2D 5'-(GCA GAT TCT GCT AAT ACC CT)-3' and 2D 5'-(AAC AGC TTT GTG CCT GGC T)-3'; 3U 5'-(CTG CAG GGT TCT AGT TTA TC)-3' and 3D 5'-(ATC AAG TGA ACT GAC AGA AG)-3'; 4U 5'-(GAC CCC CAC TCA TGT TTA GC)-3' and 4D 5'-(TTA CTT CTG CTT GGT GGC AT)-3'; 5U 5'-(GAT CTT CCA GAT AGC CCT GG)-3' and 5D 5'-(TCT TTT CAG CAG TAG GTG CTT T)-3'; 6U 5'-(AAA CAG CTC AAA CCA AGC GA)-3' and 6D 5'-(TCT GGA GTA CTT TCC GTG G)-3'; 7U 5'-(CAG AGG GTC CAG GTT CTT CC)-3' and 7D 5'-(TCC TGA ACT GGA GGC ATT ATT C)-3'. The PCR reaction conditions were follows: 5 min at 94°C and 35 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 2 min, and extension at 72°C for 1 min on a Gene Amp PCR System 9600. The size of the PCR product ranged from 126-176 bp. The electrophoresis was performed on non-denaturing 12% polyacrylamide gel and run for 1.5-3h at 300V at 15°C. After electrophoresis, the DNA was visualized by silver staining using a Silver stain plus kit (Biorad). The bands detected were cut out, and the PCR was repeated using the same primers. The re-amplified PCR products were sequenced using an automatic sequencer.

### *APC methylation analysis*

For methylation analysis, genomic DNA was modified by sodium bisulfite using a CpGenomeTM Fast DNA Modification kit (S7824; Chemicon, Temecula, CA, USA), per the manufacturer's instructions. Methylation-specific PCR (MSP) was done by a CpG WIZ APC Amplification kit (Chemicon) for the promoter 1A (GeneBank sequence No U02509), as per the manufacturer's instructions. Briefly, PCR was carried out in a 25 $\mu$l reaction mixture containing template DNA (25 ng), 1.0 $\mu$l of each primer (unmethylated and methylated), 2.5mM of each dNTP (2.5$\mu$l), 10 x PCR buffer (2.5 $\mu$l), and 0.2 $\mu$l (1U) hot start Taq polymerase (Takara). The PCR reaction conditions were as follows: 5 min at 95°C and 40 cycles of denaturation at 95°C for 30 sec, annealing at 57°C for 45 sec, and extension at 72°C for 60 sec on a Gene Amp PCR System 9600. The sizes of amplified products are 109 bp and 98 bp for unmethylated and methylated

primers, respectively.

*APC loss of heterozygosity (LOH) analysis using RFLP analysis*

To study allelic loss at exon 11 of the APC gene, the region was amplified according to the method of Uzawa et al.. Briefly, PCR was carried out in a 25 $\mu$l reaction mixture containing template DNA (25 ng), 1.0 $\mu$l of each primer, 2.5mM of each dNTP (2.5 $\mu$l), 10 $\mu$l PCR buffer (2.5 $\mu$l), and 0.2 $\mu$l (1U) of Taq polymerase (Takara). The primer sequences were as follows: sense: 5'- (GGA CTA CAG GCC ATT GCA GAA) -3' and antisense: 5'- (GGC TAC ATC TCC AAA AGT CAA) -3'. The PCR reaction conditions were as follows: 5 min at 94°C and 40 cycles of denaturation at 94°C for 1 min, annealing at 59°C for 1 min, and extension at 72°C for 1 min on a Gene Amp PCR System 9600. The size of the amplified product was 133 bp. For the RFLP analysis, 10 $\mu$l of the amplified PCR product was digested with 10 units of Rsa I restriction enzyme at 37°C for 5h. After restriction digestion, 5°C of the digest was electrophoresed on 2% agarose gel with ethidium bromide and visualized by UV transillumination.

## Results

The clinicopathologic characteristics of beta-catenin protein expression and mutational analysis based on mucin expression in ICs

ICs were divided into phenotypes as G, GI(G), GI(I), and I types based on a combination of predominant patterns of MUC5AC, MUC2, MUC6, and CD10 staining, respectively. Of the 109 cases, 32 (29.4%), 23 (21.1%), 9 (8.2%), and 45 cases (41.3%) were G, GI(G), GI(I), and I types, respectively. Fifty-four and 55 cases were ICIPs (I +GI[I] types) and ICGPs (G +GI[G] types), respectively.

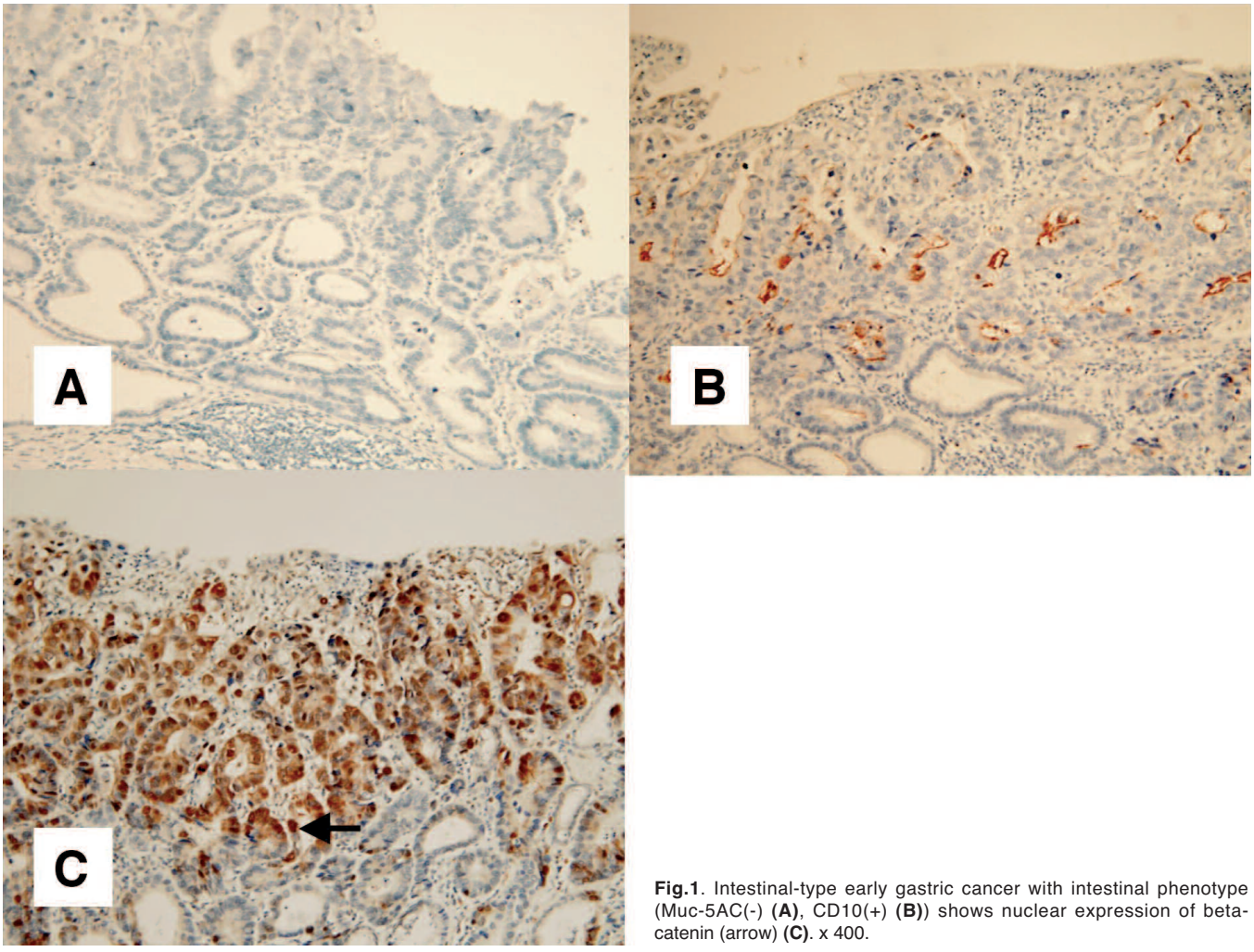


**Fig.1**. Intestinal-type early gastric cancer with intestinal phenotype (Muc-5AC(-) **(A)**, CD10(+) **(B)**) shows nuclear expression of beta-catenin (arrow) **(C)**. x 400.

Increased cytoplasmic and nuclear beta-catenin expression (delocalization) was detected in 36 (33.0%) of 109 cases of ICs. Beta-catenin delocalization was significantly higher in ICIPs (I +GI[I] types; 46.3% [25/54 cases]) than ICGPs (20% [11/55 cases]; G +GI[G] types; Table 1 and Fig. 1). Also, beta-catenin delocalization was associated with the depressed gross type. There was no statistically significant relationship with other clinicopathologic features (size, location, depth of invasion, and lymph node metastasis).

To elucidate the mechanism of beta-catenin delocalization, we performed SSCP and direct sequencing analysis for beta-catenin in 109 cases of ICs. We did not find any mobility shift in SSCP analysis, which was confirmed by direct sequencing.

### *APC mutation, promoter methylation, and LOH analysis and their relationship with beta-catenin nuclear expression and mucin phenotypes in ICs*

APC mutation analysis was performed with PCR-SSCP and direct sequencing. There was no APC mutation in 36 of 109 cases with beta-catenin delocalization on immunohistochemistry for beta-catenin. DNA from 105 ICs was evaluated for APC promoter 1A methylation using MSP. APC promoter hypermethylation was present in 49 of 105 (46.7%) cases of ICs (Fig. 2). APC promoter hypermethylation was associated with delocalization of beta-catenin in ICs (Table 2). With respect to mucin phenotypes, there was a significant relationship between APC promoter hypermethylation and beta-catenin delocalization in ICIPs, but not in ICGPs (Table 3).

For APC LOH analysis, upon digestion with Rsa I enzyme, homozygous alleles were observed as either a 133 bp fragment with no Rsa I site in the alleles, or 85 and 48 bp fragments representing the Rsa I site present in both of the alleles. The presence of heterozygosity (informative cases) in gastric normal mucosa indicated three fragments (133, 85, and 48 bp; Fig. 3). In the heterozygous group, a loss of alleles was indicated as LOH, and represented by either the 133 bp fragment or the 85 and 48 bp fragments of the APC exon 11 PCR products. We observed LOH in 30 of 69 informative cases (43.5%) in gastric cancer tissue-derived ICs (Fig. 3). Also, there was no relationship between beta-catenin delocalization and APC gene LOH (Table 2).

**Table 3.** Relationship between beta-catenin delocalization with APC promoter hypermethylation based on mucin phenotype.

| APC promoter methylation status | Case No. | Beta-catenin delocalization | | p value |
|---|---|---|---|---|
| | | Negative | Positive | |
| ICGP | | | | |
| Negative | 31 | 25 (80.6%) | 6 (19.4%) | 0.732 |
| Positive | 20 | 15(75.0%) | 5 (25.0%) | |
| ICIP | | | | |
| Negative | 25 | 17 (68.0%) | 8 (32.0%) | 0.050 |
| Positive | 29 | 12 (41.4%) | 17 (58.6%) | |

ICGP: intestinal early gastric cancer with gastric phenotypes; ICIP: intestinal early gastric cancer with intestinal phenotypes.

**Table 2.** Relationship between beta-catenin delocalization with APC promoter hypermethylation.

| | Case No. | Beta-catenin delocalization | | p value |
|---|---|---|---|---|
| | | Negative | Positive | |
| APC promoter methylation | | | | |
| Negative | 56 | 42 (75.0%) | 14 (25.0%) | 0.032 |
| Positive | 49 | 27 (55.1%) | 22 (44.9%) | |
| APC gene LOH | | | | |
| Negative | 39 | 24 (61.5%) | 15 (38.5%) | 0.624 |
| Positive | 30 | 16 (53.3%) | 14 (46.7%) | |

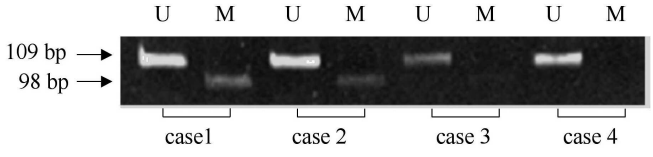


**Fig. 2.** Methylation of the APC promoter 1A is designated as methylated (M) and unmethylated (U) in intestinal-type early gastric cancer. Case 1, 2: methylated cases, case 3, 4: unmethylated cases.

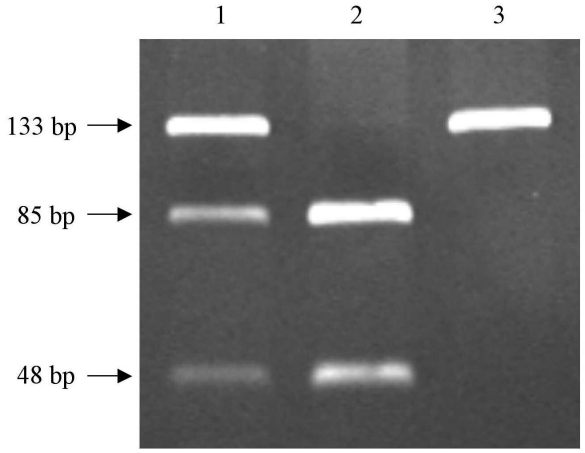


**Fig. 3.** Three kinds of electrophoretic patterns of PCR products of APC gene digested by the Rsa I enzyme. Heterozygotes show 3 bands (133 bp, 85 bp, and 48 bp) in lane 1. The digested allele is seen in the 85 and 48 bp bands (lane 2), while the undigested allele is seen as a single 133 bp band (lane 3). Loss of heterzygosity (LOH) is represented as loss of either the 133 bp fragment (lane 2) or the 85 and 45 bp fragments of the APC gene products (lane 3).

## Discussion

In this study, we found that delocalization of beta-catenin is different based on the mucin phenotypes of ICs. That is, delocalization of beta-catenin was more prominent in the intestinal mucin phenotype than the gastric mucin phenotype of ICs. Also, it was revealed that the mechanism of beta-catenin delocalization was different between ICIPs and ICGPs. APC promoter methylation might be involved in delocalization mechanisms of beta-catenin in ICIPs; however, other mechanisms might be important in beta-catenin delocalization in ICGPs.

Generally, beta-catenin may be regarded as existing in three different subcellular locations (membrane, cytosolic, and nuclear). Tyrosine phosphorylation of beta-catenin leads to dissociation of beta-catenin into the cytosol and subsequent degradation or translocation of beta-catenin into the nucleus (Wong et al., 2002). Therefore, impaired degradation of beta-catenin and increased cytosolic and nuclear beta-catenin accumulation (delocalization) can induce neoplastic transformation (Kinzler and Vogelstein, 1996; Bienz and Clevers, 2000; Satoh et al., 2000). In the current study, nuclear and cytoplasmic beta-catenin expression (delocalization) was higher in the intestinal mucin phenotype than the gastric mucin phenotype in intestinal early gastric cancer. Also, there was no relationship between the clinicopathologic features (submucosal invasion and lymph node metastasis) and beta-catenin delocalization. There are some reports pertaining to beta-catenin expression in gastric cancers. Miyazawa et al. (2000) showed nuclear expression of beta-catenin in 12% of gastric cancers, all of which were intestinal-type (differentiated) adenocarcinomas and beta-catenin nuclear expression was associated with submucosal invasion in ICs. But, Grabsch et al. (2001) suggested that beta-catenin delocalization was evident in 13.2% of gastric cancers and was not correlated with a specific histologic tumor type, tumor progression, or prognosis. Woo et al. (2001) reported that abnormal expression of beta-catenin (delocalization) was seen in 27% of gastric cancers and was significantly higher in the diffuse-type of gastric cancer. In our study, we focused on early ICs, and beta-catenin delocalization occurred in 30.0% of ICs. Interestingly, the intestinal mucin phenotype of ICs showed more beta-catenin delocalization than the gastric mucin phenotypes. There are few reports about beta-catenin expression on the basis of mucin phenotypes in gastric cancer. Aihara et al. (2005) showed that the mixed gastro-intestinal mucin phenotype was associated with nuclear expression in early undifferentiated gastric carcinoma. Ogasawara et al. (2006) reported that nuclear beta-catenin expression correlated with the intestinal phenotypic expression in gastric cancer. Thus, in conjunction with our data and previous reports, we suggest that delocalization of beta-catenin might be greater in intestinal-type gastric cancer, especially the intestinal mucin phenotypes.

With respect to the mechanisms of delocalization of beta-catenin in cancer, genetic and epigenetic alterations of various genes, including APC, beta-catenin, and AXIN, are reported to be genetic events causing impaired beta-catenin degradation and delocalization into the cytoplasm and nucleus (Kinzler and Vogelstein, 1996; Bienz and Clevers, 2000; Satoh et al., 2000). However, presumptive causative genetic alterations leading to delocalization of beta-catenin in gastric cancer are still controversial. Mutations of the beta-catenin gene are reported to be few or none in gastric cancer (Sasaki et al., 2001; Woo et al., 2001). However, there are some reports that beta-catenin mutation occurs in ICs (Park et al., 1999; Ebert et al., 2002; Ogasawara et al., 2006). In the current study, we did not identify any beta-catenin mutations in ICs. APC gene mutations have been previously reported in up to 40% of gastric adenomas, but rarely in gastric adenocarcinomas (Maesawa et al., 1995; Lee et al., 2002). We did not demonstrate LOH of the APC gene associated with beta-catenin localization. We performed PCR-RFLP methods and observed LOH in 30 of 69 informative cases (43.5%) in ICs. The frequency of APC LOH varies among investigators using different methods for LOH analysis (PCR microsatellite analysis; Wu et al., 1998; Ohmura et al., 2000). In gastric cancer, LOH on 5q with a mutation of the remaining APC gene allele leading to complete inactivation has been reported as a rare event (Horii et al., 1992; Tamura et al., 1994). We suggest that more studies are needed to confirm this contention.

Interestingly, we found that APC promoter hypermethylation has a significant association with delocalization of beta-catenin. Therefore, we suggest that loss of function of APC by APC promoter hypermethylation might be important in beta-catenin delocalization in ICs. There are several reports that APC promoter methylation occurs in gastric cancer, as well as chronic gastritis and intestinal metaplasia (Tsuchiya et al., 2000; Tamura, 2004). These data imply that APC promoter hypermethylation might have no functional effect in beta-catenin delocalization in gastric cancer. Clement et al. (2004) suggested that normal gastric mucosa showed monoallelic methylation of the APC promoter, and adjacent mucosa in gastric cancer showed altered methylation of APC promoter. They also showed that samples with a biallelic methylation pattern had a loss of APC protein in gastric tissue. Also, results differ between APC hypermethylation and beta-catenin delocalization in different tumor types (Sarrio et al., 2003; Gao et al., 2005). We suggest that altered methylation of the APC promoter might have an effect on loss of function of APC, followed by delocalization of beta-catenin. Additional studies are needed to confirm this notion. Also, there was a significant relationship between APC promoter hypermethylation and beta-catenin delocalization in ICIPs, but not in ICGPs. These data suggest that APC promoter hypermethylation is important in beta-catenin delocalization in ICIPs. In ICGPs, other mechanisms, such as AXIN or GSK-3

beta, might play roles in beta-catenin delocalization. In conclusion, we found that beta-catenin delocalization was more evident in ICIPs and APC promoter hypermethylation might play a role in delocalization of beta-catenin, especially in ICIPs.

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