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Mutation and Expression of APC/β-Catenin/E-cadherin in Colorectal Adenocarcinoma

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Abstract : The pathogenesis of colorectal adenocarcinoma is described as a sequential multistep accumulation of genetic alterations. Most research into the development of colorectal cancer has focused on the detection of mutation of particular gene, but the elucidation for the interaction of these mutated genes with cell adhesion molecules is also important to understand the colorectal tumorigenesis. To elucidate the role of APC/β-catenin/E-cadherin in the colorectal adenocarcinoma, we identified APC/β-catenin/E-cadherin mutation and expression in 42 colorectal adenocarcinomas. Mutations of APC exon 15 and β-catenin exon 3 were detected in 11 cases (26.2%) and 1 case (2.4%), but there was no evidence of aberrant shift in E-cadherin exon 6-9. APC and E-cadherin promoter methylations were found in 5 cases (11.9%) and 25 cases (59.5%). The membranous staining of APC and E-cadherin was decreased in 30 cases (71.4%) and 37 cases (88.1%). The β-catenin expression was increased in the membranes in 34 cases (81.0%), cytoplasms in 17 cases (40.0%) and nuclei in 21 cases (50.0%) . At the invasive areas of tumor growth, nuclear expression of β-catenin was common. Down-regul, β-catenin has comprehensive influence with APC and E-cadherin on tumorigenesis of colorectal adenocarcinoma. Therefore, β-catenin has comprehensive influence with APC and E-cadherin on tumorigenesis of colorectal adenocarcinoma.

Key Words : Adenocarcinoma, APC, Colon, E-cadherin, β-catenin

Introduction

APC (Adenomatous polyposis coli) tumor suppressor gene is located on chromosome 5q21[1]. A germline mutation of APC gene was first found in familial adenomatous polyposis (FAP) [2]. APC somatic mutations are known to be the earliest abnormalities in

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the development of sporadic colorectal tumor [3].

β -Catenin is a cytoplasmic protein, links E-cadherin to α-catenin, together forming the E-cadherin/catenin complex [4]. It plays a role in the organization and maintenance of epithelial integrity and in suppression of tumor invasion and metastasis [5]. Cytoplasmic β-catenin forms the complex with APC gene products, glycogen synthase kinase-3β (GSK-3β) and axin, followed by ubiquitin/proteasome degradation [6]. β-Catenin is involved in the Wingless/Wnt developmental signaling transduction pathway [7]. It also plays a role in the regulation of gene activity with a dominant oncogenic effect on tumorigenesis.

E-cadherin is a transmembrane glycoprotein, which mediates calcium-dependent intercellular adhesion that is essential for the maintenance of normal tissue architecture [8]. E-cadherin is important in the control of cellular motility in healing and carcinogenic process, morphogenesis during embryonic development, control of apoptosis associated with actin cytoskeleton, as well as formation and maintenance of adherens in cell-to-cell junction. E-cadherin expression has been widely studied in colorectal adenocarcinomas [9]. However, there has been no comprehensive study of genetic and epigenetic changes and down-regulation of E-cadherin in colorectal adenocarcinoma.

Most research into the development of colorectal cancer has focused on the detection of mutation of particular gene, but the elucidation for the interaction of these mutated genes with cell adhesion molecules is also important to understand the colorectal tumorigenesis. In this study, we examined genetic and/or epigenetic alterations and expression of APC, E-cadherin, and β-catenin in colorectal adenocarcinoma to determine the involvement of APC, E-cadherin, and β-catenin in colorectal tumor development.

Materials and Methods

1. Samples

Forty two cases of colorectal adenocarcinoma were obtained from the Department of Pathology, Keimyung University School of Medicine between 2000 and 2002. The patients included twenty one males and twenty one females, and ranged in age from 38 years to 92 years. The resected fresh tissues were divided into tumor and non-tumorous tissue, sliced as about 1 cm^3 in size, snap-frozen in the cryogenic vial and stored at liquid nitrogen tank (-135℃) for DNA studies. The tumor and non-tumorous colorectal tissues were fixed in 10% buffered formalin, and then embedded in paraffin for the routine hematoxylin and eosin stain. Immunohistochemical stains were also performed.

2. DNA extraction

DNA was extracted and purified by standard techniques from frozen tumor and non-tumorous colorectal specimens. Blocks were cut into sections and mounted on glass slides for analysis. All possible precautions were taken to avoid contamination.

3. PCR-SSCP (single strand conformation polymorphism) and sequencing

Primers used for the detection of APC, β-catenin, and E-cadherin mutations were described in Table 1. The PCR was performed as following amplification cycle. It was heated for 3 minutes at 95℃ for initial DNA denaturation, followed by 35 cycles of denaturation at 95℃ for 40 seconds, annealing at 53℃ for 40 seconds, polymerization at 72℃ for 1 minute and 20 seconds and then a final extension of 5 minutes at 72℃. All amplification procedures included DNA-free controls consisting of the amplification cocktail and distilled water in place of template DNA to check for contamination. The resultant PCR products were applied to 6% polyacrylamide (acrylamide:bis, 29:1)/Tris-borate-EDTA gels with or without 5% (v/v) glycerol and the gels were subjected to autoradiography.

4. Methylation-specific PCR (MSP)

Extracted DNA was subjected to a deamination reaction by incubation with sodium bisulfite, hydroquinone and sodium hydroxide at 50℃ for 16 hours. After removal of free bisulfite using the Wizard DNA purification resin according to the manufacturer's (Promega, Madison, USA) instruction, the modified DNAs were desulfonated with sodium hydroxide, and then the DNAs were purified by ethanol precipitation [10]. The purified DNA (about 100 ng) was amplified by PCR using primers for specific modified methylated DNA or primers for specific modified unmethylated DNA (Table 1). The products were analyzed by electrophoresis on a 4% Metaphor agarose gel (FMC, Rockland, USA).

5. Immunohistochemistry

Immunohistochemical stains were done from the formalin-fixed paraffin-embedded tissues. Polyclonal rabbit anti-human C-terminal APC (Santa Cruz Biotechnology, Santa Cruz, USA) was used at a dilution of 1:500. Monoclonal mouse anti-human-β-catenin (Transduction Laboratories, Lexington, USA) and monoclonal mouse anti-human-E-cadherin (Zymed, South San Francisco, USA) were used at a dilution of 1:100. The percentage of cells with membranous or cytoplasmic positivity was graded as follows: 0 (<5%), 1 (5-25%), 2 (26-50%), 3 (51-75%), and 4 (>75%). The staining intensity was graded as negative (0, no staining), weak (+), moderate (++), and intense (+++). Multiplication of the values for the intensity and percentage yielded a score ranging from 12 to 0. Scores of 12-9 were defined as strong staining, 8-6 as reduced, 4-1 as greatly reduced staining, and 0 as negative. For statistical reasons, scores of 12-9 were defined as preserved expression while scores 8-0 were defined as reduced expression. Nuclear staining was considered positive when more than 5% of nuclei were stained and negative when less than 5% were stained.

6. Statistical analysis

Correlations between clinicopathological findings and aberrations of APC, β-catenin, and E-cadherin were examined with the chi-square test. Data were analyzed with the SPSS system software (version 11.0).

Table 1. Sequences of Primers Used in PCR-SSCP and MSP

Primer		Sequence	Size (bp)
for PCR-SSCP			
β-Catenin	sense	5'GATTTGATGGAGTTGGACATGG3	218
(exon 3)	antisense	5'TGTTCTTGAGTGAAGGACTGAG3'	
E-cadherin			
exon 6	sense	5'TCCTCATCAGAGCTCAAGTC3'	243
	antisense	5'GGGTCCAAAGAACCTAAGAG3'	
exon 7	sense	5'TGCCCAGTCCCAAAGTGCAG3'	242
	antisense	5'TCCACACCCTCTGGATCCTC3'	
exon 8	sense	5'AGGTGGCTAGTGTTCCTGG3'	198
	antisense	5'CCTTTCTTTGGAAACCCTCTAA3'	
exon 9	sense	5'GACACATCTCTTTGCTCTGC3'	268
	antisense	5'GGACAAGGGTATGAACAGCT3'	
APC exon 15			
1	sense	5'ACTCCAATATGTTTTTCAAGATG3'	173
	antisense	5'GGAACTTCGCTCACAGGAT3'	
2	sense	5'GCAGATTCTGCTAATACCCT3'	171
	antisense	5'AACAGCTTTGTGCCTGGCT3'	
3	sense	5'CTGCAGGGTTCTAGTTTATC3'	174
	antisense	5'ATCAAGTGAACTGACAGAAG3'	
4	sense	5'GACCCCACTCATGTTTAGC3'	174
	antisense	5'TTACTTCTGCTTGGTGGCAT3'	
5	sense	5'GATCTTCCAGATAGCCCTGG3'	124
	antisense	5'TCTTTTCAGCAGTAGGTGCTTT3'	
6	sense	5'AAACAGCTCAAACCAAGCGA3'	163
	antisense	5'TCTGGAGTACTTTCCGTGG3'	
7	sense	5'CAGAGGGTCCAGGTTCTTCC3'	161
	antisense	5'TCCTGAACTGGAGGCATTATTC3'	
for MSP			
E-cadherin			
M-	sense	5'TAATTAGCGGTACGGGGGGC3'	
	antisense	5'CGAAAACAAACGCCGAATACG3'	
U-	sense	5'TTAGTTAATTAGTGGTATGG3'	
	antisense	5'ACCAAACAAAAACAAACACCAAATACA3'	
APC promotor			
1A M-	sense	5'TATTGCCCAGTGCGGGTC3'	98
	antisense	5'TCGACGAACTCCCGACGA3'	
U-	sense	5'GTGTTTTATTGTGGAGTGTGGGTT3'	108
	antisense	5'CCAATCAACAAACTCCCAACAA3'	
1B M-	sense	5'TAGAATAGCGAACGAGTGTTC3'	190
	antisense	5'TCCGACGACCACACCCCG3'	
U-	sense	5'GATAGAATAGTGAATGAGTGTTT3'	195
	antisense	5'CTTCCAACAACCACACCCCA3'	

M-: methylated; U-: unmethylated.

Results

1. PCR-SSCP and sequencing analysis

Of 42 colorectal adenocarcinomas, 11 cases (26.2%) showed aberrant shifts in exon 15 of APC(Fig. 1). One case (2.4%) showed aberrant shift in exon 3 of β-catenin (Fig. 2). Sequencing results of APC exon 15 are shown in Table 2 in detail (Table 2). Four cases showed missense mutations in codon 1512 and 1498, which was substitution of GAT (aspartic acid) by CAT (histidine), 3 cases showed missense mutations in codon 1505, which was substitution of AGC (serine) by ACC (threonine), and 2 cases showed missense mutations in codon 1318 and 1297, which was substitution of GAT (aspartic acid) by AAT (asparagine), respectively (Fig. 3). Sequencing results of β-catenin exon 3 are also shown in Table 2. Silent mutation in codon 35, which was substitution of ATC (isoleucine) by ATA (isoleucine) and missense mutation in codon 47, which was substitution of AGT (serine) by AAT

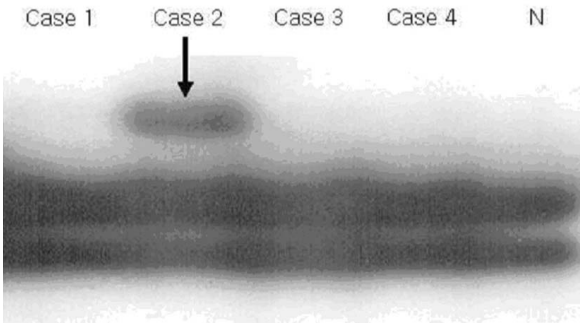

Fig. 1. Representative APC gene mutation in colorectal adenocarcinoma. Aberrant shift (arrow) in APC exon 15 was detected in case 2 of colorectal adenocarcinoma. N: non-tumorous tissue.

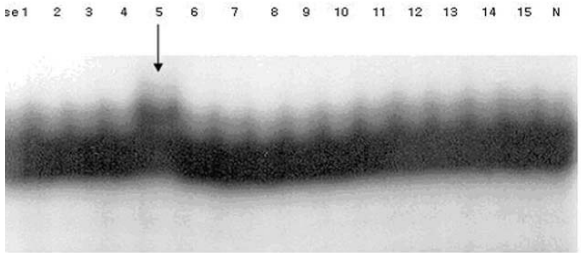

Fig. 2. β-Catenin gene mutation in colorectal adenocarcinoma. Aberrant shift (arrow) in β-catenin exon 3 was seen in case 5 of colorectal adenocarcinoma. N: non-tumorous tissue.

(asparagine) were detected (Fig. 4). There was no aberrant shift band in E-cadherin exon 6-9.

2. MSP analysis

APC promoter methylations were found in 5 cases (11.9%) (Fig. 5). E-cadherin promoter methylations were found in 25 cases (59.5%).

3. Immunohistochemical analysis

All non-tumorous colorectal glandular epithelial cells showed cytoplasmic staining of APC (Fig. 6), uniform membranous staining for β-catenin (Fig. 7) and E-cadherin (Fig. 8). There was no nuclear staining of β-catenin or E-cadherin. The membranous staining of APC and E-cadherin were absent or decreased in colorectal adenocarcinoma tumor cells than non-tumorous colorectal glandular epithelial cells in 30 cases (71.4%) and 37 cases (88.1%). The β-catenin expression was increased in colorectal adenocarcinoma tumor cells than non-tumorous colorectal glandular epithelial cells

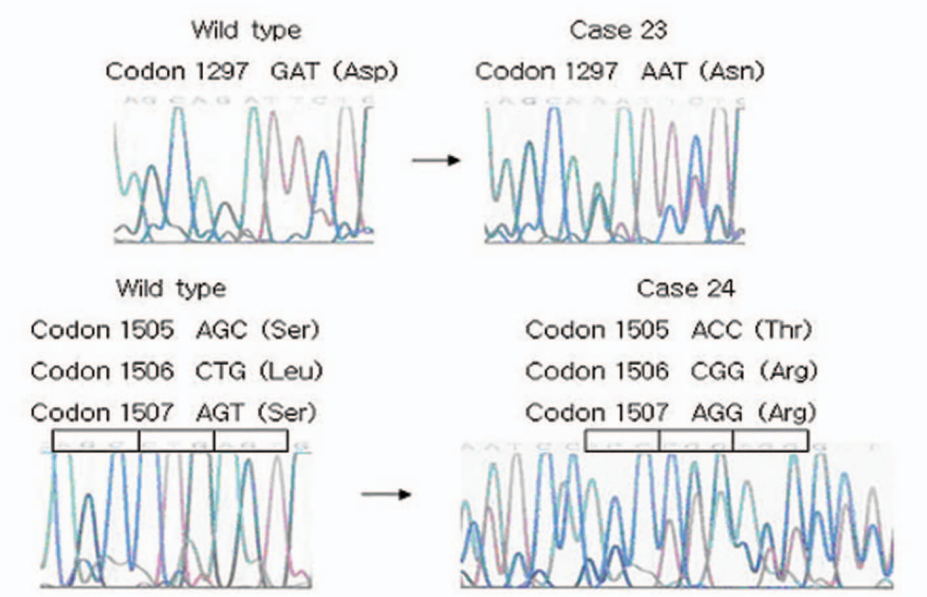

Fig. 3. Direct sequencing analysis of APC exon 15 in colorectal adenocarcinoma. Missense mutation in codon 1297 of case 23, which was substitution of GAT (aspartic acid) by AAT (asparagine) and missense mutations in codon 1505-1507 of case 24, which were substitution of AGC (serine) to ACC (threonine), CTG (leucine) to CGG (arginine), and AGT (serine) to AGG (arginine) were detected.

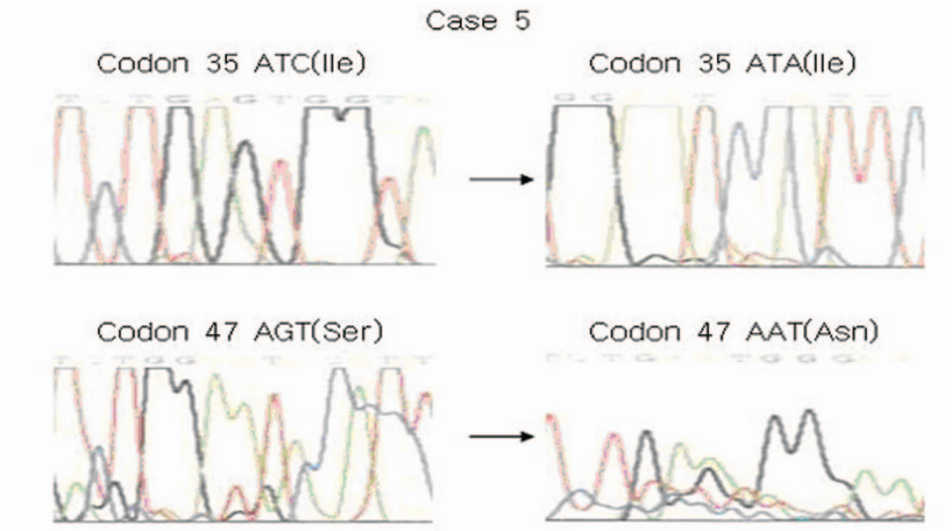

Fig. 4. Direct sequencing analysis of β-catenin exon 3 in colorectal adenocarcinoma. Silent mutation showing ATC (isoleucine) to ATA (isoleucine) transition in codon 35 and missense mutation showing AGT (serine) to AAT (asparagine) transition in codon 47 were detected.

in the membranes in 34 cases (81.0%), cytoplasms in 17 cases (40.0%) and nuclei in 21 cases (50.0%). Especially at the invasive areas of tumor growth, nuclear expression of β-catenin was common (Fig. 7).

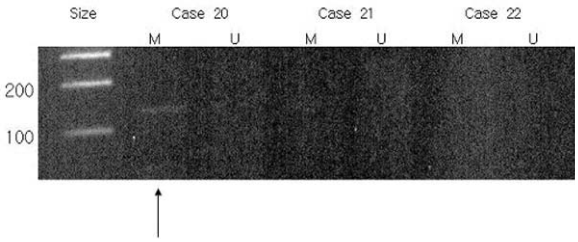

Fig. 5. Representitive methylation specific PCR for APC gene. Methylation of APC gene was found in case 20 of colorectal adenocarcinoma (arrow). M: methylated, U: unmethylated.

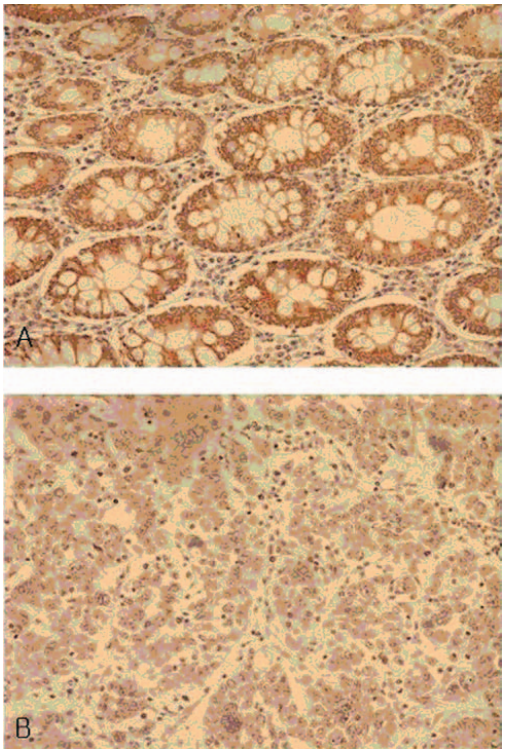

Fig. 6. Immunohistochemical stain for APC protein. A: All normal glandular epithelium showed membranous and cytoplasmic staining for APC, B: Staining of APC protein was markedly decreased in colorectal adenocarcinoma (ABC method. A-B, original magnification × 400).

4. Aberrations of APC, β-catenin and E-cadherin

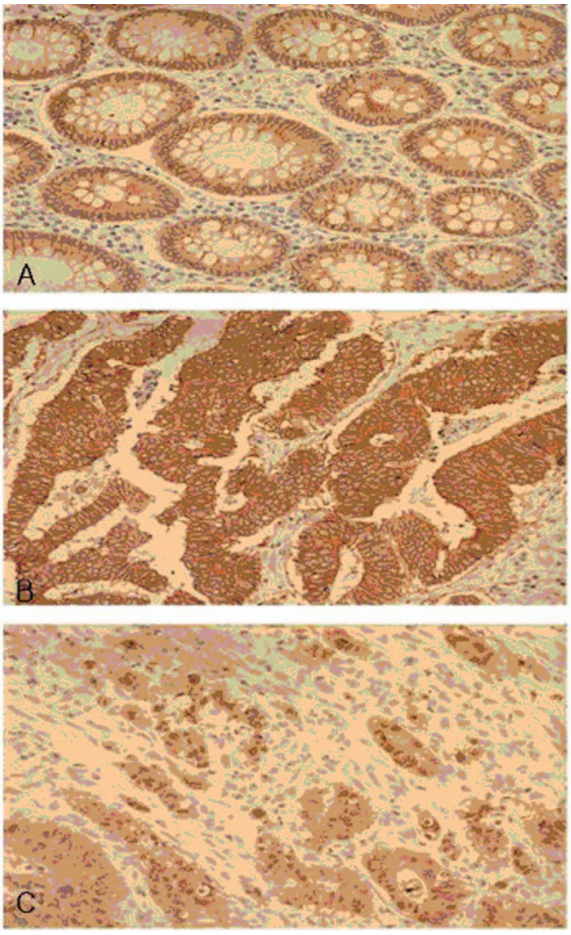

Fig. 7. Immunohistochemical stain for β-catenin protein. A: All normal epithelium showed uniform membranous staining for β-catenin, B: β-Catenin protein expression was increased in the membrane of colorectal adenocarcinoma, C: At the invasive areas of tumor growth, nuclear expression was common (ABC method. A-C, original magnification × 400).

Results of mutations, methylations and immunohistochemistry of APC, β-catenin, and E-cadherin in colorectal adenocarcinomas were summarized in Table 2. Cases with APC exon 15 mutation showed reduced APC staining in 9 cases (81.8%) out of eleven colorectal adenocarcinomas. Two cases (40%) out of 5 cases with APC promoter

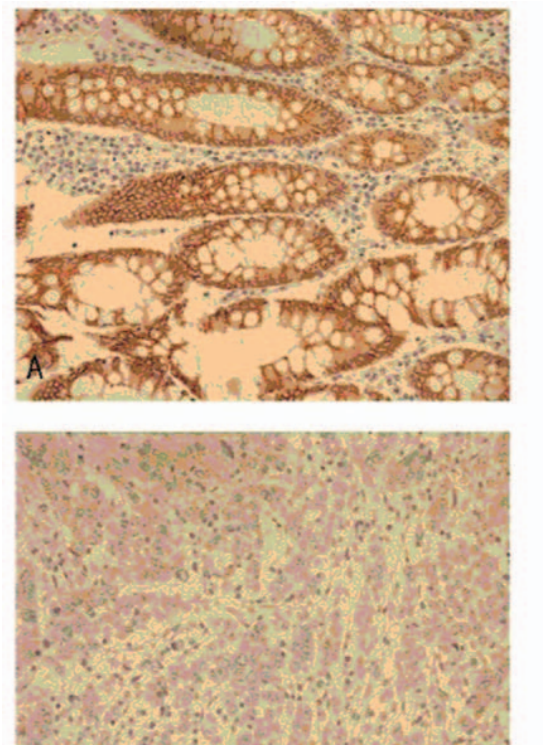

Fig. 8. Immunohistochemical stain for E-cadherin protein. A: Normal mucosal epithelium showed uniform membranous staining for E-cadherin, B: Absence of E-cadherin membranous staining was seen in colorectal adenocarcinoma (ABC method. A-B, original magnification × 400).

methylation showed reduced APC staining. Case with β-catenin exon 3 mutation showed increased β-catenin staining in all the membrane, cytoplasm, and nucleus of colorectal adenocarcinoma tumor cells. β-Catenin accumulation was found in 12 cases with APC or β-catenin mutations (100%) and 4 cases with APC promoter methylation (80%). In Table 2, all cases with reduced APC staining and 12 cases with depressed E-cadherin staining (92.3%) showed increased β-catenin staining in membrane or nucleus of colorectal adenocarcinoma tumor cells. Although E-cadherin was depressed, one case with strong APC staining showed no evidence of β-catenin accumulation in any of membrane, cytoplasm, and nucleus of tumor cells.

5. Statistical analysis

Relationship between aberrations of APC, β-catenin, and E-cadherin and its clinicopathological findings is summarized in Table 3 (Table 3). The expressions of β-catenin and E-cadherin correlated with clinical and histological parameters such as age, sex, tumor size, tumor location and lymph node involvement were not significant statistically. The parameters of vascular invasion and metastasis were not applicable with statistical analysis due to uneven distribution of data and lack of cases. The degree of E-cadherin down-regulation and the grade of tumor differentiation have statistically significant relationship ($p < 0.001$).

Discussion

APC gene lies on chromosome 5q21 and consists of 15 exons [1]. APC somatic mutations were found in 26% of sporadic colorectal carcinomas [11]. Most of the APC mutations (34-63%) occurred within a 722-base pair (bp) region (codon 1281 to 1554) of exon 15 [3]. In our study, mutations of APC exon 15 were detected in 11 cases (26.2%) of 42 colorectal adenocarcinomas, which were missense mutations with amino acid change between codon 1297 and codon 1519. Cases with APC exon 15 mutation showed reduced APC expression in 9 cases

Table 2. Summary of Mutations, Methylations and Immunohistochemistry (IHC) of APC, β-Catenin, and E-cadherin in Colorectal Adenocarcinomas

Case	APC					β-Catenin						E-cadherin
	Mutation (Exon 15)			M	IHC	Mutation (Exon 3)			IHC			IHC
	Codon	Nucleotide change (Nuc)	Amino acid change (AA)			codon	Nuc	AA	Mb	C	Nu	
2	1318	GAT → AAT	Asp → Asn	-	-		-		+	-	-	-
5				-	-	35 47	ATC → ATA AGT → AAT	Ile → Ile Ser → Asn	+	+	+	-
10	1519	GAT → GAA	Asp → Glu	-	-		-		+	+	+	+
15	1507	AGT → AAT	Ser → Asn	-	-		-		+	-	+	-
16	1512	GAT → CAT	Asp → His	-	+		-		+	+	+	-
19	1512	GAT → CAT	Asp → His	+	+		-		-	+	+	-
20		-		+	+		-		-	-	-	-
23	1297	GAT → AAT	Asp → Asn	-	-		-		+	-	+	-
24	1505 1506 1507	AGC → ACC CTG → CGG AGT → AGG	Ser → Thr Leu → Arg Ser → Arg	-	-		-		+	-	-	+
28				+	+		-		+	-	+	-
30	1505 1512	AGC → ACC GAT → CAT	Ser → Thr Asp → His	-	-		-		+	+	+	-
32	1505	AGC → ACC	Ser → Thr	-	-		-		+	+	+	-
35	1498 1502 1513	GAT → CAT TGT → TGG GAG → GAA	Asp → His Cys → Trp Glu → Glu	-	-		-		+	+	+	-
38				+	+		-		+	+	+	+
39				+	-		-		-	-	+	-
41	1503	TCA → GCA	Ser → Ala	-	-		-		+	-	-	-

M: methylation; Mb: membranous staining; C: cytoplasmic staining; Nu: nuclear staining; +: positive or expression level similar to non-tumorous colorectal glandular epithelial cells; -: negative or reduced staining.

(81.8%) and increased β-catenin expression in all of 11 colorectal adenocarcinomas. Most of APC mutations result in truncated APC protein [12]. Loss of APC function for degradation of β-catenin results in the accumulation of β-catenin [13]. Experimental evidence that reintroduction of wild-type APC results in decreased β-catenin levels was reported [12]. We also found APC promoter methylations in 5 cases (11.9%) of

Table 3. Relationship between Clinicopathological Findings and Aberrations of APC, β-Catenin, and E-cadherin in 42 Colorectal Adenocarcinomas

		Number	APC		β-Catenin		E-cadherin	
			-	+	-	+	-	+
Age	< 60 yr	21	13	8	4	17	7	14
	≥ 60 yr	21	17	4	4	17	8	13
Sex	Male	21	14	7	6	15	8	13
	Female	21	16	5	2	19	7	14
Location	Right	9	5	4	4	5	4	5
	Left	33	15	8	4	29	11	22
Tumor grade	WD & MD	33	25	8	2	31	7	26
	PD	9	5	4	6	3	8	1
Tumor size	< 5 cm	22	18	4	2	20	6	16
	≥5 cm	20	12	8	6	14	9	11
Vascular invasion	Absent	6	4	2	1	5	1	5
	Present	36	26	10	7	29	14	22
Lymph node invasion	Absent	21	17	4	4	17	7	14
	Present	21	13	8	4	17	8	13
Metastasis	Absent	39	29	10	8	31	15	24
	Present	3	1	2	0	3	0	3

+: well preserved or strong membranous stain; -: distinctly or slightly decreased membranous stain;
WD: well differentiated; MD: moderately differentiated; PD: poorly differentiated.

42 colorectal adenocarcinomas. Cases with APC promoter methylation showed reduced APC expression in 2 cases (40%) and increased β−catenin expression in 4 cases (80%). It suggests that the inactivation of APC gene may be caused by a second mutation or other mechanism such as methylation [3]. Therefore, alterations of APC/β−catenin pathway are mostly caused by inactivation of the APC gene, which is early genetic event in sporadic colorectal carcinogenesis [14].

β−Catenin gene maps to 3p21 [5]. Normal cells have little free β−catenin proteins, and most of them are bound to cadherin. The rest form the complex with APC gene products, GSK−3β, and axin [6]. β−Catenin mutations were reported in nearly half of colorectal tumors lacking APC mutations [15]. But in our study, mutation of β−catenin exon 3 was detected in only one case (2.4%) of 42 colorectal adenocarcinomas. Rare β−catenin

mutation may not be the main cause of altered β-catenin expression in colorectal adenocarcinomas. The mutation site of β-catenin exon 3 was responsible for GSK-3β phosphorylation site, which also showed increased β-catenin expression. Using Western blot analysis, β-catenin levels were approximately three times higher in familial colorectal tumors than in corresponding normal tissue [13]. Undetected causes for β-catenin abnormality may be mutation outside the region studied, epigenetic silencing, or alteration of other proteins involved in the APC/β-catenin signaling cascade.

E-cadherin gene is located on 16q23 and consists of 16 exons [16]. E-cadherin is composed of extra- and intra-cellular domains. The extracellular domain is essential for the maintenance of normal tissue architecture [8]. The intracellular domain binds to β-catenin (or γ-catenin) and forms the complex (E-cadherin-catenin unit) with α-catenin, which binds to the actin cytoskeleton. In gastrointestinal tumors, loss of E-cadherin due to deletions of E-cadherin gene was reported frequently [17]. In our study, although aberrant shift in E-cadherin exon 6-9 was not detected, E-cadherin promoter methylations were found in 25 cases (59.5%). This result suggests that one of the mechanisms for loss of E-cadherin expression in sporadic colorectal cancer is gene silencing by hypermethylation of the promoter region.

β-Catenin is multifunctional protein that involved in cadherin-mediated cell-cell adhesion and the Wingless/Wnt signaling pathway [15]. β-Catenin dysregulation appears secondary to APC inactivation and phosphorylated β-catenin can modulate the E-cadherin/catenin complex [18]. In our study, nuclear expression of β-catenin was common at the invasive areas of tumor growth. Accumulated β-catenin translocates into the nucleus, which is correlated with nuclear localization of β-catenin protein. Increased cytoplasmic and/or nuclear expression of β-catenin in colorectal adenocarcinoma results in disturbed cadherin-dependent cell adhesion and increased invasive potential [19]. APC and E-cadherin were known to control cell motility and regulate cell adhesiveness. Down-regulation of APC and E-cadherin also results in increased β-catenin expression and affects dissociation of cells due to loosened intercellular adhesion [20]. Therefore, β-catenin expression is largely regulated by its two major binding partners, E-cadherin on the membrane and APC protein in the cytoplasm. In previous study, reduced E-cadherin expression in colorectal cancer was associated with a poorly differentiated phenotype, infiltrative growth, lymph node involvement, tumor recurrence and mortality [20]. However, all this findings were not confirmed in our study due to uneven distribution of data and lack of cases. The degree of E-cadherin down-regulation was correlated with the grade of tumor differentiation. Further evaluation for relationship between clinicopathological features and aberrations of these molecules may be helpful to understand the carcinogenic mechanism and to predict the prognosis in colorectal adenocarcinoma.

Several immunohistochemical studies for the expression of β-catenin and E-cadherin in colorectal tumors have investigated [7,21]. β-Catenin expression was increased in

cytoplasms and nuclei of sporadic colorectal cancers and E-cadherin expression was decreased in membranes of sporadic colorectal cancers [21]. However, the relationship of APC, β-catenin and E-cadherin during tumorigenesis remains unclear. In our results, membranous staining of APC and E-cadherin were decreased in 30 cases (71.4%) and 37 cases (88.1%) of 42 colorectal adenocarcinomas. β-Catenin expression was increased in 34 cases (81.0%). APC inactivation caused by APC mutation or gene silencing such as methylation was related with reduction of APC expression. It was confirmed in Western blot analysis as well as immunohistochemistry in sporadic colorectal tumors [22]. β-Catenin mutation induced increased expression of β-catenin protein in colorectal adenocarcinomas. E-cadherin expression was decreased in colorectal carcinomas without E-cadherin mutation. It was also proven that transcriptional silencing of E-cadherin by promoter methylation leads to down-regulation of E-cadherin expression in some tumors [23]. Down-regulation of APC and E-cadherin by genetic mutation or epigenetic change such as methylation as well as β-catenin mutation was closely related with increased expression of β-catenin protein in colorectal adenocarcinoma. Increased β-catenin expression is explained by secondary change arising from APC inactivation or competitive binding of β-catenin to APC and E-cadherin than by carcinogenic effect directly involved in tumorigenesis of colorectal adenocarcinoma. But undetected β-catenin mutation or epigenetic silencing may be involved and accumulated β-catenin may produce the unpredictable neoplastic growth by transcriptional activation of other gene promoter. β-Catenin expression can be controlled by other factors such as Wingless-type (Wnt) frizzled proteins and signaling cascade (GSK-3β, TCF/LEF-1) [24], or abnormal cross-talk with other signaling pathways [25]. In the future, the comprehensive molecular studies for these factors or cross talk of signaling pathways should be done to find out whether accumulated β-catenin is one of independent carcinogenic factors directly involved in tumorigenesis of colorectal adenocarcinoma, or secondary dysfunction arising from other carcinogenic mechanism such as APC inactivation, or additional phenomenon occurred after down-regulation of APC and E-cadherin due to competitive binding of β-catenin.

Summary

In this study, to elucidate the role of APC/β-catenin/E-cadherin in the colorectal adenocarcinomas, the authors identified APC/β-catenin/E-cadherin mutation and expression in forty two colorectal adenocarcinomas using PCR-SSCP, methylation-specific PCR, and immuno-histochemical stain. Mutations of APC exon 15 and methylations of APC promoter were found in 26.2% and 11.9% of 42 colorectal adenocarcinomas. APC inactivation caused by APC mutation or epigenetic silencing such as methylation was related with reduction of APC expression. β-Catenin exon 3 mutation was detected in 2.4%, which increased β-catenin expression. Although there was no evidence of aberrant shift in E-cadherin exon

6-9, E-cadherin promoter methylations were found in 59.5%, which decreased E-cadherin expression. Down-regulation of APC and E-cadherin by genetic mutation or epigenetic change such as methylation as well as β-catenin mutation was closely related with increased expression of β-catenin protein in colorectal adenocarcinoma. Expression of APC and E-cadherin were decreased in 71.4% and 88.1%. β-Catenin expression was increased in 81.0%. Nuclear expression of β-catenin was common at the invasive areas of tumor growth. The degree of reduced E-cadherin expression and the grade of tumor differentiation were statistically significant. Increased β-catenin expression is caused by secondary change arising from APC inactivation or competitive binding of β-catenin to APC and E-cadherin as well as β-catenin mutation. Therefore, β-catenin has comprehensive influence with APC and E-cadherin on tumorigenesis of colorectal adenocarcinoma.

References

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