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Inverse Relationship between *APC* Gene Mutation in Gastric Adenomas and Development of Adenocarcinoma

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Gastric cancer is common among the world, but genetic mechanisms of gastric carcinogenesis are not well understood. Gastric polypoid adenomas and flat dysplasias are regarded as precursor lesions. However, a detailed molecular study of these lesions has not been done to determine their role as precancerous lesions. We investigated mutations of the *APC*, *β-catenin*, and *K-ras* genes, and microsatellite instability (MSI) status in 35 adenomas and 47 flat dysplasias without adenocarcinoma, 35 adenomas/dysplasias associated with adenocarcinomas, and 39 adenocarcinomas (20 diffuse type and 19 intestinal type). Somatic *APC* gene mutations were identified in 76% (59 of 78) of adenomas or flat dysplasias without associated adenocarcinoma, but in only 3% (1 of 30) of adenomas/dysplasias associated with adenocarcinoma, and in only 4% (3 of 69) of adenocarcinomas ($P < 0.000001$). No mutations of *β-catenin* were found in adenocarcinomas, or adenomas/dysplasia without *APC* mutation. *K-ras* mutations were detected in 5% (4 of 82) of gastric adenomas/dysplasia without carcinoma, 3% (1 of 39) of adenocarcinomas without associated adenoma/dysplasia, and not in 32 adenocarcinomas with associated adenoma/dysplasia. High level of MSI (MSI-H) was more frequent in gastric adenoma/dysplasia associated with carcinoma (17%, 6 of 35) than in adenomas/dysplasia without carcinoma (3%, 2 of 75; $P = 0.01$). MSI-H was also more frequent in intestinal type adenocarcinoma (20%, 11 of 54) than in diffuse type (0%, 0 of 20; $P = 0.03$). *APC* gene mutations were present in six of nine (67%) of gastric adenomas/dysplasias with low level of MSI, but in none of the eight adenomas/dysplasia with MSI-H phenotype ($P = 0.009$). Our results indicate that somatic mutation of the *APC* gene plays an important role in the pathogenesis of gastric adenoma and dysplasia but has a limited role in neoplastic progression to adenocarcinoma. Gastric adenomas or dysplasias without *APC* mutations but with or without MSI may have a different biological behavior, and are precursors of intestinal-type of gastric adenocarcinomas. *(Am J Pathol 2002, 161:611–618)*

Gastric cancer is one of the most common malignant neoplasms among the world. Genetic alterations have been shown to play roles in gastric carcinogenesis including abnormalities in proto-oncogenes (*K-ras* and *β-catenin*), tumor suppressor genes (*p53* and *APC*), cell-cycle regulator genes (*E-cadherin*), tissue invasion-related genes (CD44), and mismatch repair genes (*hMLH1*).[1–13] Gastric adenomas and dysplasias are considered precursor lesion of invasive gastric cancer, but the genetic mechanisms of early gastric carcinogenesis are not well understood.

There are two major histological types of gastric adenocarcinoma (intestinal and diffuse) according to the Lauren classification.[14] The pathogenesis and genetic alterations for these two distinct types of adenocarcinoma are also different.[15–17] The most frequent gastric malignancy is the intestinal type, which is often preceded by sequential steps of precancerous changes, including atrophic gastritis, intestinal metaplasia, and either dysplasia or adenoma. In contrast, the diffuse type of gastric carcinoma tends to arise *de novo* and is infrequently associated with dysplasia or adenoma.[18–21] Abnormal expression and amplification of the *Met* gene, inactivation of the *p53* tumor suppressor gene, abnormal transcription of CD44, and loss of telomerase are common events in both types.[15–17,22,23] Reduced or absent expression of E-cadherin and *K-sam* gene amplification are unique to the diffuse type gastric adenocarcinoma.[16,17,22] By contrast, *K-ras* mutations, *c-erb2* gene amplification, mutations of the *APC* gene, allelic loss of *Bcl*II gene and *DCC* locus, and microsatellite instability (MSI, replication error) are preferentially associated with the intestinal-type.[16,17,22] Gastric adenoma (polypoid dysplastic mucosal lesion) and flat dys-

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plasia have been regarded as precancerous lesions for intestinal type adenocarcinoma. The sequential accumulation of alternations of *APC* and *K-ras* genes, characteristic of the colorectal adenoma-carcinoma sequence, however, does not frequently occur between adenoma and intestinal-type adenocarcinoma of the stomach.[3–7,24–26]

There are two lines of evidence indicating that not all gastric dysplastic lesions are precursor lesions for gastric carcinoma. First, gastric dysplasia can undergo spontaneous regression clinically, especially low-grade dysplastic lesions.[27,28] Only 11 to 40% of adenoma/dysplasia progress to carcinoma.[29–31] Secondly, *APC* mutations have been reported to occur more frequently in gastric adenomas than in gastric adenocarcinomas.[24–26] However, the *APC* mutation status of gastric adenoma/dysplasia lesions associated with adenocarcinomas has not been studied in detail. It is not clear whether the *APC* gene plays any role in the pathogenesis of adenocarcinomas arising from pre-existing adenoma/dysplasia. Furthermore, genetic alterations separating these two distinct morphological precancerous lesions (flat dysplasia and polypoid adenoma) remain unclear. In this study, we investigated genetic alterations in gastric adenomas, flat dysplasias, adenocarcinoma with associated adenoma/dysplasia, and adenocarcinoma without associated adenoma/dysplasia to determine their potential roles in gastric carcinogenesis.

Materials and Methods

Case Selection

This study included 35 adenomas and 47 flat dysplasias from endoscopic biopsies of patients without gastric carcinoma, 39 adenocarcinomas (20 diffuse type and 19 intestinal type) without an associated adenoma/dysplasia, and 35 adenocarcinomas associated with adenoma/dysplasia (29 adenomas/dysplasias were immediately adjacent to the carcinoma, and 6 were distant) from surgical resection specimens (approved by University of Texas MD Anderson Cancer Center Institutional Review Board). All of the cases were retrospectively identified from the surgical pathology files of Chonnam National University Hospital, Kwangiu, South Korea between 1996 to 2000. No patient had familial adenomatous polyposis (FAP). The distinction between adenoma and dysplasia was based on endoscopic findings. Gastric adenoma was defined as a polypoid, elevated or exophytic lesion, and flat dysplasia as a flat or depressed lesion. Gastric adenomas and dysplasias were subclassified in hematoxylin and eosin (H&E)-stained slides into low-grade and high-grade dysplasia according to published criteria.[32] The carcinomas were classified histologically according to Lauren[14] and staged according to the criteria of the International Union Against Cancer.[33] Location and size of tumor were also recorded. Gastric nonneoplastic mucosa was evaluated for the presence of intestinal metaplasia, mucosal atrophy by Sydney criteria, and *Helicobacter pylori* by Giemsa stain and Campylobacter-like organism (CLO) test.[34] The clinicopathogical features of patients are summarized in Table 1. Most of adenocarcinomas associated with adenoma/dysplasia were stage 1 tumors and intestinal type carcinomas.

DNA Extraction

Microdissection from formalin-fixed and paraffin-embedded tissue was performed on H&E-stained slides for both tumor and normal mucosa. A 271/2-gauge needle was used for microdissection of H&E-stained slides under a low-power (×4) objective. In cases of adenocarcinoma associated with adenoma/dysplasia, carcinoma and adenoma/dysplasia components were separately microdissected and analyzed. Genomic DNA was extracted from microdissected tissue as described previously.[35]

APC Gene Mutation Analysis

Four sets of oligonucleotide primers (5′-CAGACTTATTGTGTAGAAGA-3′ and 5′-CTCCTGAAGAAAATTCAACA-3′ for codons 1260 to 1350; 5′-AGGGTTCTAGTTTATCTTCA-3′ and 5′-TCTGCTTGGTGGCATGGTTT-3′ for codons 1339 to 1436; 5′-GGCATTATAAGCCCCAGTGA-3′ and 5′-AAATGGCTCATCGAGGCTCA-3′ for codons 1417 to 1516; 5′-ACTCCAGATGGATTTTCTTG-3′ and 5′-GGCTGGCTTTTTTGCTTTAC-3′ for codons 1497 to 1596) were used to amplify the mutation cluster region of the *APC* gene for gastrointestinal tumors. Polymerase chain reaction (PCR) was performed under standard conditions in a 50-μl volume using PCR Master (Boehringer Mannheim, Mannheim, Germany) and 1 μmol/L of both 5′ and 3′ oligonucleotides with 40 cycles (94°C for 1 minute, 58°C for 1 minute, and 72°C for 2 minutes). PCR products were purified using shrimp alkaline phosphatase and exonuclease I (Amersham, Buckinghamshire, UK). Purified PCR products were sequenced directly with SequiTherm Excel II DNA Sequencing Kit (Epicentre, Madison, WI) with the same primers used for DNA amplification. Oligonucleotides were end-labeled with [γ-^{32}P]-ATP (DuPont-New England Nuclear Research Products, Boston, MA) using T4 polynucleotide kinase (New England Biolabs, Beverly, MA). All mutations were verified in both the sense and anti-sense directions.

β-Catenin Gene Mutation Analysis

Mutation analysis of the *β-catenin* gene was performed only on cases that did not show detectable *APC* mutations. Genomic DNA from each sample was amplified by PCR using the forward primer 5′-ATGGAACCAGACAGAAAAGC-3′ and reverse primer 5′-GCTACTTGTTCTTGAGTGAAG-3′. These amplified a 200-bp fragment of exon 3 of the *β-catenin* gene encompassing the region for GSK-3β phosphorylation. PCR reaction was performed under standard conditions in a 50 μl volume using PCR Master (Boehringer Mannheim) and 1 μmol/L of both 5′ and 3′ oligonucleotides with 40 cycles (94°C for 1 minute, 58°C for 1 minute, and 72°C for 2 minutes). PCR products were purified and sequenced directly using internal primers (forward, 5′-AAAGCGGCTGTTAGTCACTGG-3′; re-

Table 1. Clinicopathological Characteristics of Gastric Adenoma, Dysplasia, and Adenocarcinoma

	Adenocarcinoma (−)		Adenocarcinoma (+)	
	Adenoma	Dysplasia	with Adenoma/dysplasia	without Adenoma/dysplasia
Case no.	35	47	35	39
Age (mean ± SD)	67 ± 9.5	61 ± 8.3	63 ± 8.1	59 ± 11.3
Sex				
Male	28	37	27	22
Female	7	10	8	17
Location				
Body	22	19	17	22
Antrum	13	28	18	17
Size (mm)				
≤10	10	21	9	8
11–20	14	16	12	8
≥21	11	10	14	23
Tumor size (mm)				
Mean ± SD	20.7 ± 15.8	17.8 ± 11.4	21.3 ± 11.1*	35.4 ± 24.8*
Stage				
I			34†	13†
II			1†	4†
III			0†	15†
IV			0†	7†
Histologic grade				
Low grade	24	24		
High grade	11	23		
Histologic subtype				
Intestinal			35‡	19‡
Diffuse			0‡	20‡
Intestinal metaplasia				
Present	27	36	31	28
Absent	8	11	4	11
Mucosal atrophy				
Present	11§	28§	28	35
Absent	24§	19§	7	4
H. pylori infection				
Present	23	30	32	31
Absent	12	17	3	8

*$P = 0.003$; †$P < 0.001$; ‡$P < 0.001$; §$P = 0.01$.

verse, 5′-CCTGTTCCCACTCATACAGG-3′) as described above. All mutations were verified in both the sense and anti-sense directions.

K-ras Oncogene Mutation Analysis

The first exon of *K-ras* was amplified in a 50-μl volume using the reaction mixture described above with oligonucleotide primers (5′-GGCCGGTAGTGTATTAACCTTATGTGTGACAT-3′ and 5′-CCGCGGCCGGCGGCCAAAACAAGATTTACCTCTATTGTTGG-3′). PCR products were purified as described. The purified PCR products were sequenced using ABI PRISM BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) with an internal primer 5′-ATTCGTCCACAAAATGAT-3′. The sequencing reactions were run on an ABI PRISM 3700 DNA Analyzer (Applied Biosystems). The data were collected and analyzed using Applied Biosystems sequencing software, according to the manufacturer's protocols.

Microsatellite Instability Analysis

MSI status was determined by five fluorescently labeled PCR amplifications using fluorescent dye-labeled forward primer and unlabeled reverse primer (BAT-25, BAT-26, D2S123, D5S346, and D17S250). The forward oligonucleotide was end-labeled with 6-FAM (Applied Biosystems). PCR was performed in 15-μl volumes containing 40 ng of DNA, 9 μl ABI Prism True Allele PCR Premix (Applied Biosystems), 5 pmol of 6-FAM-labeled forward primer, and 10 pmol of unlabeled reverse primer. PCR was performed using the following cycling conditions: denaturation at 95°C for 6 minutes, 45 cycles of 94°C for 45 seconds, 55°C for 45 seconds, 72°C for 1 minute, and extension at 72°C for 30 minutes. The PCR product was diluted further with 30 μl of H_2O, and a 1.0 μl aliquot of each diluted fluorescent-labeled PCR product was combined with 12 μl of formamide and 0.5 μl of Genescan 400HD (ROX) size standard (Applied Biosystems). The samples were then capillary electrophoresed on an ABI 3700 DNA Analyzer using GeneScan Analysis software (Applied Biosystems). Allelic shift (MSI) of a microsatellite marker was defined by the presence of at least one additional band in the DNA from tumor or invasive carcinoma that was not present in the control normal DNA. A specimen was considered as MSI-high (MSI-H) when at least two markers showed allelic shift or MSI-low (MSI-L) when only one marker was shifted.

Table 2. *APC*, *β-catenin*, and *K-ras* Mutations in Gastric Adenomas, Flat Dysplasias, and Adenocarcinomas

	APC	*β-catenin*	*K-ras*
Adenoma/dysplasia without carcinoma (n = 82)	76% (59/78)*†	0% (0/19)	5% (4/82)
Polypoid adenoma (n = 35)	77% (24/31)	0% (0/7)	9% (3/35)
Flat dysplasia (n = 47)	74% (35/47)	0% (0/12)	2% (1/47)
Adenoma/dysplasia associated with carcinoma (n = 35)	3% (1/30)*	0% (0/35)	0% (0/32)
Adenocarcinoma (n = 74)	4% (3/69)†	0% (0/74)	1% (1/71)
Intestinal type (n = 54)	4% (2/49)	0% (0/54)	2% (1/51)
with adenoma/dysplasia (n = 35)	7% (2/30)	0% (0/35)	0% (0/32)
without adenoma/dysplasia (n = 19)	0% (0/19)	0% (0/19)	5% (1/19)
Diffuse type (n = 20)	5% (1/20)	0% (0/20)	0% (0/20)

*P <0.000001; †P < 0.000001.

Statistical Analysis

Fisher's exact test was used to compare differences in clinical or pathological characteristics, gene mutation, and MSI phenotype. A P value of <0.05 was considered statistically significant.

Results

Somatic Mutations of APC, β-Catenin, and K-ras Genes

Somatic *APC* mutations were more frequent in gastric adenomas and flat dysplasia without carcinoma (76%, 59 of 78) than in adenocarcinomas (4%, 3 of 69; P < 0.000001) or in adenomas/dysplasias associated with adenocarcinoma (3%, 1 of 30; P < 0.000001) (Table 2). There was no difference in the *APC* mutation rate between polypoid adenomas (77%, 24 of 31) and flat dysplasias (74%, 35 of 47). Similarly, there was no difference in the *APC* mutation rate between adenocarcinomas associated with adenoma/dysplasia (7%, 2 of 30) and adenocarcinomas without associated adenoma/dysplasia (3%, 1 of 39). Two adenocarcinomas associated with adjacent adenoma/dysplasia had *APC* mutations. One was a frameshift mutation (4-bp deletion of TCTC spanning codons 1464 to 1465) observed in both the carcinoma and adenoma/dysplasia components, and the other was a frame shift mutation (1-bp insertion of A in a poly(A) tract spanning codons 1554 to 1556) detected only in the carcinoma component not in the adenoma/dysplasia component. There was no correlation between the presence of *APC* mutation and grade of dysplasia in flat dysplasia or adenoma (88% and 76% in low grade *versus* 61% and 80% in high grade, respectively, P = 0.4). The distribution of *APC* mutations is summarized in Figure 1. Seventy-nine percent (50 of 63) of *APC* mutations were frameshifts (26 deletions and 24 insertions), and 21% (13 of 63) were nonsense point mutations resulting in truncation of the APC gene product. An insertion of A into the poly(A) tract at codons 1554 to 1556 was the most common (42%, 21 of 50) of the frameshift mutations. There were no significant associations between *APC* mutations and other clinicopathological parameters. No *β-catenin* mutations were detected in adenocarcinomas (n = 74), adenomas (n = 10), or dysplasia (n = 14) without *APC* mutation.

K-ras mutations were detected in 5% (4 of 82) of gastric adenomas/dysplasia without carcinoma, and 3% (1 of 39) of adenocarcinoma without associated adenoma/dysplasia, but were not found in adenocarcinoma with associated adenoma/dysplasia (n = 32). Only one gastric adenoma had both an activating codon 12 *K-ras*

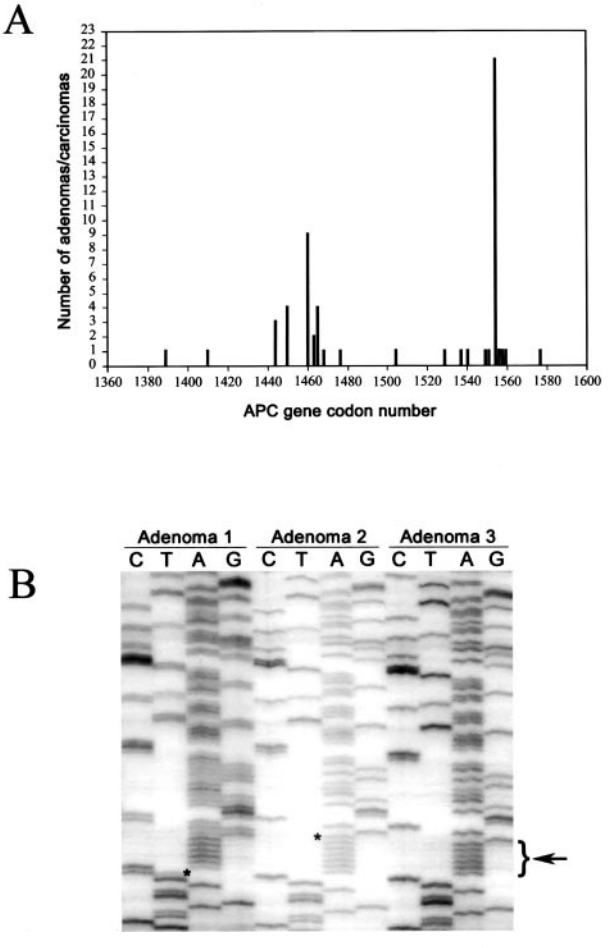

Figure 1. Somatic *APC* gene mutations in gastric adenomas/dysplasias and adenocarcinomas. **A:** Summary of the *APC* gene mutations in the mutation cluster region. An insertion of A into the poly(A) tract at codons 1554 to 1556 is the most common mutation. **B:** An insertion of T (**asterisk**) at codon 1556 (adenoma 1), and an insertion of A (**asterisk**) into the poly(A) tract (**bracket with arrow**) at codons 1554 to 1556 (adenoma 2). In adenoma 2, the absence of normal sequence also indicates loss of the nonmutated *APC* allele. Adenoma 3 has normal *APC* sequence.

Table 3. *APC* Mutation and Microsatellite Instability (MSI) Status in Gastric Tumors with *K-ras* Mutation

No.	Histologic grade/type	*K-ras* mutation			*APC* mutation	MSI status
		Codon	Nucleotide change	Amino acid change		
1	Dysplasia, HG	12	GGT→GAT	Gly→Asp	–	MSI-S
2	Adenoma, LG	12	GGT→GCT	Gly→Ala	ND	MSI-L
3	Adenoma, HG	12	GGT→GAT	Gly→Asp	+	MSI-L
4	Adenoma, HG	12	GGT→GCT	Gly→Ala	ND	MSI-S
5	Adenocarcinoma intestinal-type	12	GGT→GAT	Gly→Asp	–	MSI-H

HG, high grade; LG, low grade; ND, not done; MSI-S, MSI stable phenotype; MSI-L, MSI-low phenotype; MSI-H, MSI-high phenotype.

mutation and a frame shift mutation of the *APC* gene (Table 3).

Microsatellite Instability

MSI-H phenotype was more frequent in gastric adenoma/dysplasia associated with carcinoma (17%, 6 of 35) than in adenoma/dysplasia without carcinoma (3%, 2 of 75; $P = 0.01$) (Table 4). Two of 30 adenomas, but none of 45 flat dysplasias had MSI-H phenotype. MSI-H phenotype was also more frequent in intestinal-type adenocarcinoma (20%, 11 of 54) than in diffuse-type (0%, 0 of 20; $P = 0.03$). There was no difference in the frequency of MSI-L phenotype between gastric adenoma/dysplasia associated with carcinoma (9%, 3 of 35) and adenomas/dysplasia without carcinoma (9%, 7 of 75); or between intestinal type adenocarcinoma and diffuse type [6% (3 of 54) *versus* 5% (1 of 20), respectively].

MSI phenotype was not correlated with grade of dysplasia. Among two MSI-L flat gastric dysplasias, one was high-grade dysplasia and one was low-grade dysplasia. Similarly, among two MSI-H and five MSI-L adenomas, three were high-grade dysplasia and four were low-grade dysplasia. Among adenocarcinomas associated with adenoma/dysplasia, MSI+ phenotype was present in both the carcinoma and adenoma/dysplasia components in five cases (four MSI-H and one MSI-L), in carcinoma alone in four cases (two MSI-H and two MSI-L), and in adenoma/dysplasia alone in four cases (two MSI-H and two MSI-L). Three of five MSI+ cases had a different allelic shift pattern between the carcinoma and adjacent adenoma/dysplasia components (Figure 2).

Correlation between APC, K-ras, and Microsatellite Instability Status

There was an inverse correlation between the presence of MSI-H and the presence of *APC* gene mutations. *APC* gene mutations were present in six of nine (67%) gastric adenomas/dysplasias with MSI-L phenotype, but in none of the eight adenomas/dysplasia with MSI-H phenotype ($P = 0.009$) (Table 5). The three MSI-L adenomas/dysplasias without *APC* mutations were associated with adenocarcinomas. No *APC* mutations were detected in any of the 15 MSI+ (11 MSI-H and 4 MSI-L) gastric adenocarcinomas.

There was no association between *K-ras* mutation and MSI phenotype. Among five tumors with *K-ras* mutations, two were MSI-S, two were MSI-L, and one was MSI-H (Table 3).

Discussion

Gastric dysplastic lesions such as polypoid adenomas or flat dysplasia are frequently seen in patients with chronic atrophic gastritis and intestinal metaplasia. Intestinal-type gastric carcinomas also typically arise in a background of atrophic gastritis with intestinal metaplasia.[18] Although gastric carcinomas can arise from pre-existing dysplastic lesions, only a subset of gastric carcinoma has identifiable preneoplastic lesions.[29–31] The adenoma-carcinoma sequence typically seen in colorectal carcinoma does not seem to be a major pathway in gastric carcinogenesis. This issue is further complicated by the

Table 4. Microsatellite Instability (MSI) in Gastric Adenoma/Dysplasia and Adenocarcinoma

	MSI (+)	MSI-H	MSI-L
Adenoma/dysplasia without carcinoma ($n = 82$)	12% (9/75)	3% (2/75)*	9% (7/75)
Polypoid adenoma ($n = 35$)	23% (7/30)	7% (2/30)	17% (5/30)
Flat dysplasias ($n = 47$)	4% (2/45)	0% (0/45)	4% (2/45)
Adenoma/dysplasia associated with carcinoma ($n = 35$)	26% (9/35)	17% (6/35)*	9% (3/35)
Adenocarcinoma ($n = 74$)			
Intestinal type ($n = 54$)	26% (14/54)	20% (11/54)†	6% (3/54)
with adenoma/dysplasia ($n = 35$)	26% (9/35)	17% (6/35)	9% (3/35)
without adenoma/dysplasia ($n = 19$)	26% (5/19)	26% (5/19)	0% (0/19)
Diffuse type ($n = 20$)	5% (1/20)	0% (0/20)†	5% (1/20)

*$P = 0.01$; †$P = 0.03$.
MSI (+) includes both MSI-H and MSI-L phenotypes; MSI-H, MSI high phenotype; MSI-L, MSI low phenotype.

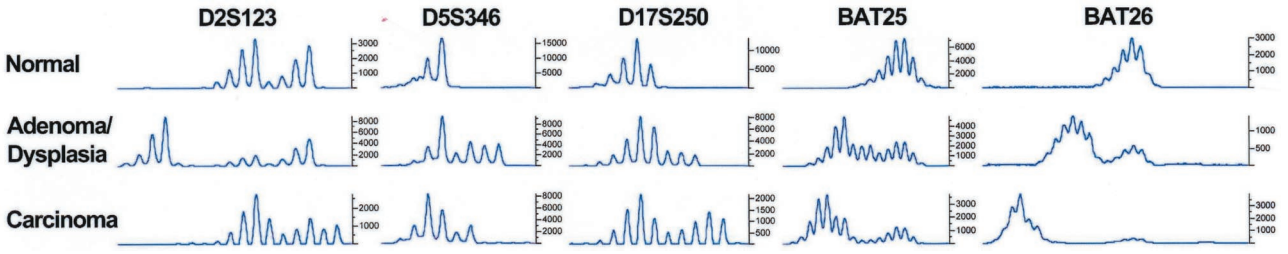

Figure 2. Microsatellite instability in gastric adenocarcinoma and the associated adenoma/dysplasia. The adenoma/dysplasia component shows a different allelic shift pattern in all five markers (D2S123, D5S346, D17S250, BAT25, and BAT26) as compared to the adenocarcinoma component.

facts that only a small subset of gastric adenoma or dysplastic lesions eventually progress to carcinoma.

The reason for lack of neoplastic progression in the majority of gastric adenomas and dysplastic lesions is not clear. *APC* gene mutations have been previously reported in up to 40% of gastric adenomas but only rarely in gastric adenocarcinomas.[3,4,24–26,36,37] The presence of frequent somatic *APC* mutations in gastric adenoma but not in gastric carcinoma suggests that there is a different genetic pathway for the pathogenesis of adenoma and carcinoma. However, in previous studies only isolated adenomas and gastric carcinomas have been studied. Gastric adenoma/dysplasia lesions associated with adenocarcinomas have not been studied in detail for the presence of *APC* mutation.[3,4,25,26,36] In most cases, the adenocarcinoma can be assumed to have arisen within the dysplasia or adenoma. If the *APC* gene plays any role in the pathogenesis of adenocarcinomas arising from pre-existing adenoma/dysplasia, *APC* gene mutations should be detected in both adenoma/dysplasia and adenocarcinoma components, similar to the frequency of *APC* gene mutations in adenomas or dysplasias without adenocarcinoma. Our study demonstrates that *APC* mutations are frequent in sporadic gastric adenomas and dysplasias, but only rarely in adenocarcinomas. This rare presence of *APC* mutations was true for both adenocarcinomas with or without associated adenoma/dysplasia. The findings in this study therefore strongly indicate that *APC* gene mutations play an important role in the pathogenesis of gastric adenoma and dysplasia, but have only a limited role in the pathogenesis of gastric adenocarcinomas. *β-catenin* gene mutations have previously been reported in 5% (4 of 77) to 16% (7 of 43) of gastric adenocarcinomas from the Korean population.[8,38] In contrast, we found no *β-catenin* gene mutations in 54 intestinal and 20 diffuse type adenocarcinomas. The reason for this discrepancy is not clear.

Table 5. *APC* Gene Mutation Status in MSI+ Gastric Adenoma/Dysplasia

	MSI-H	MSI-L
APC Gene Mutation		
Present	0% (0/8)*	67% (6/9)*
Absent	100% (8/8)*	33% (3/9)*

*$P = 0.009$.
MSI-H, microsatellite instability high phenotype; MSI-L, microsatellite instability low phenotype.

Our results also suggest that *APC* gene mutation status could predict the biological behavior of individual gastric adenoma/dysplasia lesions (Figure 3). Gastric adenomas/dysplasias with *APC* gene mutations only rarely progress to adenocarcinoma. This corroborates the clinical observation that only 11 to 40% of adenomas/dysplasias can progress to carcinoma.[29–31] This is contradictory to the role of the *APC* gene in colorectal carcinogenesis, in which it is regarded as a gatekeeper gene and is involved in the vast majority of colorectal carcinomas.[39] Because we only sequenced the mutation cluster region of the *APC* gene, we cannot exclude *APC* mutations outside this region. It is possible that *APC* gene mutations in adenocarcinomas either associated or not associated with adenoma/dysplasia are different from sporadic gastric adenomas or dysplasias, and are not present within the mutation cluster region we have sequenced. However, the presence of only rare *APC* gene mutations in gastric carcinomas in previously published studies further corroborates our results.[40] In addition, we have previously shown frequent somatic second-hit of *APC* genes in FAP-associated gastric fundic gland polyps.[41] Foveolar dysplasia can present in up to 25% of FAP-associated fundic gland polyps, but rare occurrence of adenocarcinomas in FAP-associated fundic gland polyps. Therefore, *APC* gene mutations seem to have a different biological behavior in the stomach as compared to the colorectum, and gastric adenomas/dysplasia with *APC* mutations only rarely progress to adenocarcinomas.

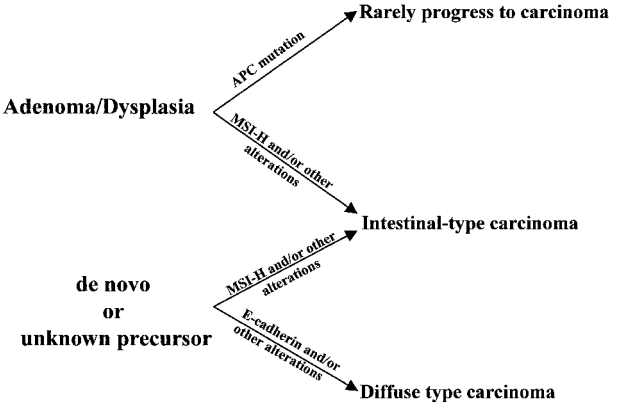

Figure 3. Role of *APC* gene and MSI in gastric adenoma/dysplasia-carcinoma sequence. Adenocarcinoma can arise *de novo* or from pre-existing adenoma/dysplasia. Adenomas or dysplasias with *APC* gene mutations rarely progress to adenocarcinoma, and have a different biological behavior as compared to adenomas or dysplasias with MSI.

Gastric dysplastic lesions can be divided into adenomas (with a polypoid configuration) or flat dysplasias based on growth pattern.[32] However, it is difficult to differentiate adenoma from flat dysplasia once adenocarcinoma has arisen from these pre-existing dysplastic lesions. Furthermore, genetic alterations of these two distinct morphological gastric dysplastic lesions (polypoid adenoma and flat dysplasia) have not been studied in detail. We found no difference in the frequency of *APC* mutations in gastric adenomas *versus* flat dysplasias. In the colorectum, *K-ras* mutations are associated with a polypoid growth pattern in colorectal adenomas and adenocarcinomas, and are more frequent in polypoid adenomas as compared to flat adenomas.[42–44] In contrast, in our study the prevalence of *K-ras* mutations was not different between polypoid adenomas and flat dysplasias in the stomach.

Microsatellite instability (MSI) because of DNA replication errors has been widely observed in a variety of sporadic tumors in addition to tumors associated with hereditary nonpolyposis colorectal cancer syndrome because of germ line mutations in mismatch repair genes.[45–48] MSI has been identified in 7 to 50% of gastric carcinomas with geographic variation in prevalence.[49] We found MSI-H phenotype in 11% (8 of 75) of gastric adenomas/dysplasias and in 20% (11 of 54) of gastric intestinal type adenocarcinomas, which are similar to previously published results; 21% (13 of 63) in gastric adenomas and 30% (19 of 63) in gastric carcinomas from the Korean population.[46] Similar to colorectal carcinomas, MSI-positive gastric carcinomas have distinct clinicopathological features including better prognosis, and prominent lymphoid infiltration. MSI-positivity is more common in intestinal type gastric carcinoma located in the antrum than in diffuse type carcinoma.[50–53] In the present study, we found a higher prevalence of MSI-H phenotype in adenoma/dysplasia associated with adenocarcinoma than adenomas not associated with adenocarcinoma, as previously reported.[54] Three of five MSI+ adenocarcinomas associated with adenoma/dysplasia components had a different allelic shift pattern between carcinoma and adjacent adenoma/dysplasia components. One explanation for this finding is that an adenocarcinoma acquires a different allelic size during progression of tumor from adenoma/dysplasia. This phenomenon has been described in MSI+ tumor cells.[55] However, the possibility of adenocarcinoma arising *de novo*, and not from the pre-existing adenoma/dysplasia cannot be completely excluded. MSI+ colorectal and pancreatic carcinomas have a distinct genetic profile with wild-type *K-ras* and lack of *p53* gene mutations.[55–57] Similarly, MSI-H gastric adenocarcinomas or adenomas/dysplasias also correlated with the absence of *APC* gene mutations in our study. In contrast, MSI-L gastric adenomas/dysplasias had frequent *APC* mutations.

In conclusion, our results demonstrate that somatic mutation of the *APC* gene plays an important role in the pathogenesis of gastric adenoma and dysplasia but has limited role in neoplastic progression to adenocarcinoma. In contrast, gastric adenomas or dysplasias without *APC* mutations may have a different biological behavior, and are precursors of intestinal type of gastric adenocarcinomas. MSI-H can play an important role in a subset of adenocarcinomas arising from gastric adenomas/dysplasias without *APC* mutations.

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