PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 June 28; 12(24): 3803-3809
World Journal of Gastroenterology ISSN 1007-9327

Tumor differentiation phenotype in gastric differentiated-type tumors and its relation to tumor invasion and genetic alterations

Kimiyasu Yamazaki, Yusuke Tajima, Reiko Makino, Nobukazu Nishino, Shigeo Aoki, Masanori Kato, Masaaki Sakamoto, Koji Morohara, Tsutomu Kaetsu, Mitsuo Kusano

Kimiyasu Yamazaki, Yusuke Tajima, Reiko Makino, Nobukazu Nishino, Shigeo Aoki, Masanori Kato, Masaaki Sakamoto, Koji Morohara, Tsutomu Kaetsu, Mitsuo Kusano, Department of Surgery, Division of General and Gastroenterological Surgery, Showa University, School of Medicine, 1-5-8, Hatanodai, Shinagawa-ku, Tokyo 142-8666, Japan
Reiko Makino, Clinical Research Laboratory, Showa University, School of Medicine, 1-5-8, Hatanodai, Shinagawa-ku, Tokyo 142-8666, Japan
Supported by Grant-in-Aid for Scientific Research, Japan Society for the Promotion of Science, No. 17790928
Correspondence to: Yusuke Tajima, Department of Surgery, Division of General and Gastroenterological Surgery, Showa University, School of Medicine, 1-5-8, Hatanodai, Shinagawa-ku Tokyo 142-8666, Japan. surgery@med.showa-u.ac.jp
Telephone: +81-3-37848541 Fax: +81-3-37845835
Received: 2005-12-29 Accepted: 2006-01-14

Abstract

AIM: To clarify the relations between tumor differentiation phenotype and tumor invasion or genetic alterations in gastric differentiated-type tumors.

METHODS: We examined the tumor differentiation phenotype, the presence of mutations in *APC* and *p53*, and the microsatellite instability (MSI) status in 48 gastric adenomas and 171 differentiated-type carcinomas. The tumor differentiation phenotype was determined by examining the expression of human gastric mucin (HGM), MUC6, MUC2 and CD10. The tumors were then classified into gastric- (G-), gastric and intestinal mixed- (GI-), or intestinal- (I-) phenotypes, according to the immunopositivity of the above markers. The presence of mutations in *APC* and *p53* and the MSI status were also investigated in all the tumors.

RESULTS: Gastric adenomas were significantly associated with CD10 expression, I-phenotype tumors and the presence of *APC* mutations, compared with carcinomas (66.7% *vs* 25.1%, $P < 0.0001$; 56.3% *vs* 14.6%, $P < 0.0001$; 39.6% *vs* 14.0%, $P < 0.0001$, respectively) and inversely associated with expressions of HGM and MUC6 and the presence of *p53* mutations (10.4% *vs* 62.6%, $P < 0.0001$; 39.6% *vs* 64.3%, $P = 0.003$; 2.0% *vs* 26.3%, $P = 0.001$, respectively). The frequency of *APC* mutations was significantly higher in HGM-negative tumors, MUC6-negative tumors, CD10-positive tumors and I-phenotype tumors than in HGM-positive tumors, MUC6-positive tumors, CD10-negative tumors and G-phenotype tumors (32.7% *vs* 7.1%, $P < 0.0001$; 27.8% *vs* 14.0%, $P = 0.0182$; 37.3% *vs* 10.4%, $P < 0.0001$; and 38.5% *vs* 9.5%, $P = 0.0017$, respectively). The frequency of MSI was significantly higher in MUC6-positive tumors, CD10-negative tumors and G-phenotype tumors than in MUC6-negative tumors, CD10-positive tumors and I-phenotype tumors (24.8% *vs* 6.7%, $P = 0.0009$; 22.2% *vs* 8.0%, $P = 0.0143$; and 28.6% *vs* 9.6%, $P = 0.0353$, respectively).

CONCLUSION: The tumor differentiation phenotype is closely related to tumor invasion and genetic alterations in gastric differentiated-type tumors.

Key words: Gastric carcinoma; Tumor differentiation phenotype; *APC*; *p53*; Microsatellite instability

Yamazaki K, Tajima Y, Makino R, Nishino N, Aoki S, Kato M, Sakamoto M, Morohara K, Kaetsu T, Kusano M. Tumor differentiation phenotype in gastric differentiated-type tumors and its relation to tumor invasion and genetic alterations. *World J Gastroenterol* 2006; 12(24): 3803-3809

http://www.wjgnet.com/1007-9327/12/3803.asp

INTRODUCTION

Gastric carcinoma is histologically classified into two types, differentiated- and undifferentiated-type or intestinal- and diffuse-type, based on the prevalent gland formation[1,2]. The pathogenesis and genetic alterations leading to these two distinct types of adenocarcinoma are generally considered different. Previous molecular genetic studies have shown that carcinogenesis is a multistep process characterized by accumulating genetic alterations[3,4]. *K-ras* mutations, *c-erbB-2* amplification, *APC* mutations, allelic loss of the *Bcl*-II and *DCC* loci, and microsatellite instability (MSI) are preferentially associated with differentiated-type carcinoma[5,6]. Conversely, the reduced or absent expression of *E-cadherin* and *K-sam* amplification are unique to undifferentiated-type carcinoma. The abnormal expres-

sion and amplification of *Met*, the inactivation of the *p53* tumor suppressor gene, the abnormal transcription of *CD44*, and the loss of telomerase are common events in both types[3,4,7]. Recently, several authors have reported that gastric carcinomas can be classified as having either a gastric- (G-), gastric and intestinal mixed- (GI-) or intestinal- (I-) phenotype, depending on the immunopositivity of human gastric mucin (HGM), MUC6, MUC2 and CD10 stainings[8-10]. HGM, MUC6, MUC2 and CD10 are specifically expressed in gastric surface mucous cells, pyloric gland cells, intestinal goblet cells of the mature gastrointestinal tract, and the brush border of intestinal epithelial cells, respectively. We previously reported that G-phenotype tumors account for 27.7% of differentiated tumors, and are often referred to as intestinal-type tumors according to Lauren, while I-phenotype tumors account for 10.1% of undifferentiated tumors[8]. The phenotypic marker expression of tumors is conventionally thought to imitate that of the tissue of origin[11-13]. Thus, the above data suggest that gastric carcinomas can occur in various types of gastric mucosa, although differentiated tumors are generally thought to arise from gastric mucosa with intestinal metaplasia while undifferentiated tumors are thought to arise from ordinary gastric mucosa without intestinal metaplasia[1,2]. Recently, G-phenotype tumors have been associated with a poorer outcome and greater malignant potential, compared with tumors of other phenotype[8-10,14-17]. Therefore, this phenotypic classification is useful not only for investigating the tumorigenesis of gastric carcinoma, but also for evaluating tumor aggressiveness. Phenotypic expression in gastric neoplasm has also been suggested to depend on genetic changes[18]. However, there are conflicting opinions regarding the relations between tumor differentiation phenotype and tumor invasion or genetic alterations in gastric differentiated-type tumors.

In the present study, we examined the tumor differentiation phenotype, mutations in *APC* and *p53* genes, and the MSI status in 48 gastric adenomas and 171 differentiated-type adenocarcinomas to clarify the relations between tumor differentiation phenotype and tumor invasion or genetic alterations in gastric differentiated-type tumors.

MATERIALS AND METHODS

Patients

The present series consisted of 48 patients with gastric adenoma having undergone an endoscopic biopsy or an endoscopic mucosal resection and 171 patients with differentiated-type gastric adenocarcinoma having undergone surgical resection between 2001 and 2003 at Showa University Hospital. Written informed consent was obtained from all the patients before they were included in the present study.

Histological review

The specimens were fixed with 10% buffered formalin and embedded in paraffin. Consecutive sections (4 μm thick) were stained with hematoxylin and eosin (HE) and immunohistochemical stains for the histopathological examination. The intramucosal neoplastic lesions were histologically classified according to the general rules established by the Vienna classification[19]. Forty-eight adenomas (35 low-grade adenomas and 13 high-grade adenomas), 132 differentiated-type, early-stage carcinomas (80 intramucosal carcinomas and 52 carcinomas with invasion to the submucosal layer), and 39 differentiated-type, advanced-stage carcinomas (13 carcinomas with invasion to the muscularis propria layer, 15 carcinomas with invasion to the subserosa, and 11 carcinomas with invasion to the serosa) were identified.

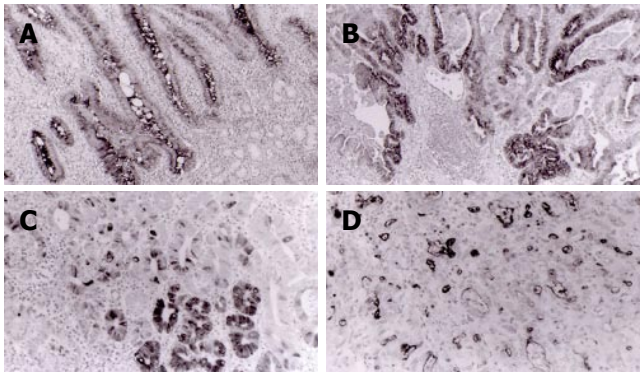

Figure 1 Immunohistochemical analysis of tumor differentiation phenotype in gastric tumor. **A**: Human gastric mucin (HGM) (45M1, × 200); **B**: MUC6 (CLH5, × 100); **C**: MUC2 (Ccp58, × 200); **D**: CD10 (56C6, × 200).

Analysis of tumor differentiation phenotype

The following mouse monoclonal antibodies were used: 45M1 (Novocastra Laboratories Ltd, UK) diluted 1:50 to detect HGM, CLH5 (Novocastra Laboratories Ltd, UK) diluted 1:50 to detect MUC6 glycoprotein, Ccp58 (Novocastra Laboratories Ltd, UK) diluted 1:100 to detect MUC2 glycoprotein, and 56C6 (Novocastra Laboratories Ltd, UK) diluted 1:40 to detect CD10 glycoprotein expression. 45M1 and CLH5 were examined as gastric-phenotype markers, and Ccp58 and 56C6 were examined as intestinal-phenotype markers. 45M1 could recognize the mucin epitope located in the peptide core of HGM, synonymous with MUC5AC. This antibody is known to react with surface foveolar cells in the stomach[20] (Figure 1A). MUC6 glycoprotein is expressed in mucous cells in the neck zone of oxyntic mucosa and in antral glands[21,22] (Figure 1B). MUC2 glycoprotein, also known as the "intestinal-mucin related protein antigen", is an intestinal apomucin that is known to be expressed in the supranuclear area of goblet cells in regions showing intestinal metaplasia in the stomach[22,23] (Figure 1C). CD10 glycoprotein is a 100-kDa cell metalloendopeptidase that inactivates a variety of biologically active peptides and is known to be expressed on the brush border of intestinal epithelial cells as well as in the germinal centers of lymphoid follicles[24] (Figure 1D). The avidin-biotin-peroxidase complex immunohistochemical method was used for all immunohistchemical studies according to a previously described protocol[25].

With regard to the evaluations of HGM, MUC6, MUC2, and CD10 staining, distinct staining in more than 5% of the tumor cells was recorded as positive immunore-

	Gastric-phenotype markers HGM - MUC6 +	
Intestinal-phenotype markers MUC2 -		Gastric-phenotype
Intestinal-phenotype markers CD10 +	Intestinal-phenotype	Gastric and intestinal mixed-phenotype

Figure 2 Classification of tumor differentiation phenotype. The phenotypes were classified into three groups (gastric-phenotype, intestinal-phenotype, gastric and intestinal mixed-phenotype) according to the combination of the expression of human gastric mucin (HGM), MUC6, MUC2 and CD10.

activity for the relevant marker. These immunohistochemical methods were used to classify the tumors into three different phenotypes: tumors with gastric phenotypic cells accounting for more than 5% of their cell population were classified as gastric- (G-) phenotype tumors, those with intestinal phenotypic cells accounting for more than 5% of their cell population were classified as intestinal- (I-) phenotype tumors, and those with both gastric and intestinal phenotypic cells accounting for more than 5% of their cell population were classified as gastric and intestinal mixed- (GI-) phenotype tumors as previously described[8,9,26] (Figure 2).

DNA extraction

Serial sections (10 μm thick) of formalin-fixed, paraffin-embedded tumor and normal mucosa were stained with HE and precisely microdissected under microscopic visualization using a PixCell laser capture microdissection system (Arcus Engineering, Mountain View, CA) to avoid DNA contamination from inflammatory or stromal cell nuclei and the DNA of cancerous cells inconsistent with the overt differentiation phenotype of the tumor. Genomic DNA was extracted from the microdissected tissue as previously described[27].

Mutation analysis of APC and p53

APC mutations in the exon 15, codons 1260-1596 region were detected by fluorescence-based PCR-single-strand conformation polymorphism analysis (PCR-SSCP) using an automated DNA sequencer as previously described[28]. This region was amplified by 4 parts using the following primer sets: 5'-CAGACTTATTGTGTAGAAGA-3' and 5'-CTCCTGAAGAAAATTCAACA-3', 5'-AGGGTTC-TAGTTTATCTTCA-3' and 5'-TCTGCTTGGTGGCAT-GGTTT-3', 5'-AACCATGCAGTGGAATGGTA-3' and 5' -AAATGGCTCATCGAGGCTCA-3', 5'-ACTCCAGAT-GGATTTTCTTG-3' and 5'-GGCTGGCTTTTTTGCTT-TAC-3'. The 5'-terminals of the primers were labeled with Cy5 dye. DNA fragments were amplified by PCR from 2 μL of 50 μL of extracted DNA. PCR was performed at 94℃ for 1 min followed by 40 cycles at 94℃ for 1 min, at 58℃ for 1 min, and at 72℃ for 2 min. Mutations were detected by electrophoresis using the ALFexpress DNA sequencer (Amersham Biosciences, NJ, USA) with TME buffer (30 mmol/L Tris-HCl, 36 mmol/L MES, 1 mmol/L EDTA; pH 6.8) at 20℃, 25℃, and 30℃. The detected peaks were analyzed using the Fragment Manager program (Amersham Biosciences).

p53 mutations in the exon 5-8 region were detected by fluorescence-based PCR-SSCP using capillary electrophoresis[28]. The nucleotide sequences of the forward and reverse primers were as follows: 5'-TTCACTTGT-GCCCTGACTTTC-3' and 5'- CTCTCCAGCCCCAGCT-GCTC-3' for exon 5, 5'-ATTCCTCACTGATT-GCTCTT-3' and 5'-TCCTCCCAGAGACCCCAGTT-3' for exon 6, 5'-ACAGGTCTCCCCAAGGCGCA-3' and 5'-TGCAGGGTGGCAAGTGGCT-3' for exon 7, 5' -GGTAGGACCTGATTTCCTTACTGCC-3' and 5' -CCCTTGGTCTCCTCCACCGCTTCTTG-3' for exon 8. The 5'-terminals of the primers for p53 were labeled with FAM. DNA fragments were amplified by PCR from 2 μL of 50 μL of extracted DNA. Mutations were detected by electrophoresis using a 3100 genetic analyzer (Applied Biosystems, CA, USA) with 2 × TME buffer (60 mmol/L Tris-HCl, 72 mmol/L MES, 2 mmol/L EDTA). The precise electrophoresis conditions have been described elsewhere[29]. The peak pattern was analyzed using QUISCA software, established by Higasa *et al*[30] and kindly provided by Dr. K Hayashi. The nucleotide sequences of DNA fragments with shifted peaks were determined using a genetic analyzer 310 with a BigDye Terminator (Applied Biosystems).

Microsatellite instability analysis

Microsatellite instability was detected using five microsatellite markers: BAT-25, BAT-26, D2S123, D5S346, and D17S250. The primers and PCR conditions have been described elsewhere[31]. The 5'-terminals of the forward primers were labeled with FAM. PCR was performed at 94℃ for 1 min followed by 35 cycles at 95℃ for 30 s, at 50℃ for 1 min, and at 70℃ for 1 min. The samples were capillary electrophoresed on an ABI 3100 genetic analyzer using Genescan analysis software (Applied Biosystems). An allelic shift (MSI) in a microsatellite marker was identified by the presence of at least one additional band in the tumor's DNA that was not present in the control DNA. A specimen was defined as MSI-high (MSI-H) when at least two markers showed instability, MSI-low (MSI-L) when only one marker showed instability, and microsatellite stable (MSS) when none of the markers exhibited MSI[32].

Statistical analysis

The data were analyzed using Student *t*-test or Mann-Whitney *U*-test and chi square test or Fisher' exact test, as appropriate. $P < 0.05$ was considered statistically significant.

RESULTS

HGM, MUC6, MUC2 and CD10 expressions, tumor differentiation phenotype, and genetic alterations in 219 gastric tumors

The expression of HGM, MUC6, MUC2, and CD10 was demonstrated in 112 (51.1%), 129 (58.9%), 163 (74.4%) and 75 (34.2%) of the 219 gastric tumors, respectively. Taking into account the observed combinations of these phenotypic markers, the 219 tumors were classified into 42 G-phenotype tumors (19.2%), 125 GI-phenotype

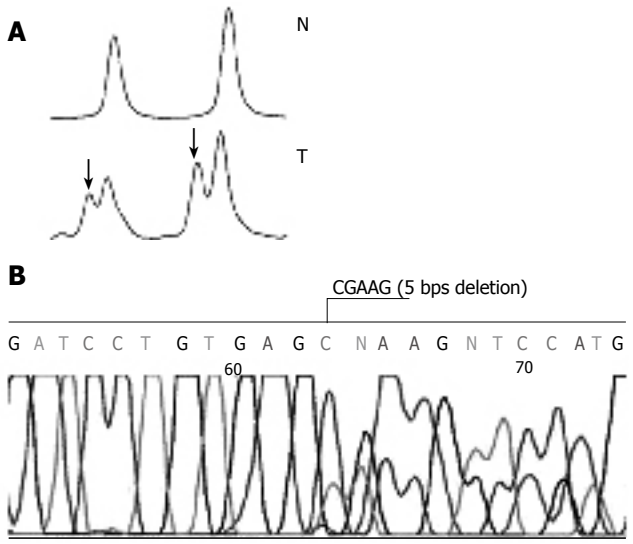

Figure 3 Mutation analysis of the APC gene in gastric tumour. **A**: PCR-single-strand conformation polymorphism (PCR-SSCP) analysis shows shift peak (arrowhead) as compared with the control normal DNA; **B**: DNA sequence analysis reveals frameshift mutation (CGAAG, 5 bps deletion) at codons 1321 of the APC gene. T: Tumor DNA, N: Normal DNA.

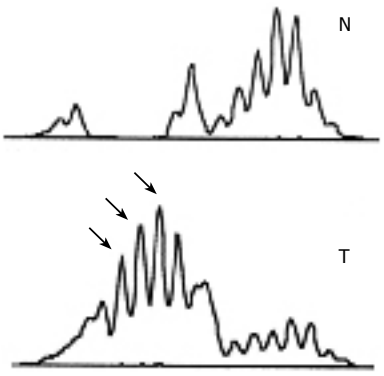

Figure 4 Microsatellite instability (MSI) using marker BAT-26 shows a different allelic shift peak (arrowhead) as compared with the control normal DNA. T: Tumor DNA, N: Normal DNA.

Table 1 Phenotypic expressions, tumor differentiation phenotype and genetic alterations in gastric adenoma and carcinoma *n* (%)

Phenotypic expressions, tumor differentiation phenotype &genetic alterations	Adenoma (*n* = 48)	Early carcinoma (*n* = 132)	Advanced carcinoma (*n* = 39)	Early and advanced carcinoma (*n* = 171)
HGM				
Negative	43 (89.6)	49 (37.1)[d]	15 (38.5)[d]	64 (37.4)[d]
Positive	5 (10.4)	83 (62.9)[d]	24 (61.5)[d]	107 (62.6)[d]
MUC6				
Negative	29 (60.4)	48 (36.4)[b]	13 (33.3)[a]	61 (35.7)[b]
Positive	19 (39.6)	84 (63.6)[b]	26 (66.7)[a]	110 (64.3)[b]
MUC2				
Negative	6 (12.5)	35 (26.5)	15 (38.5)[a]	50 (29.2)[a]
Positive	42 (87.5)	97 (73.5)	24 (61.5)[a]	121 (70.8)[a]
CD10				
Negative	16 (33.3)	100 (75.8)[d]	28 (71.8)[c]	128 (74.9)[d]
Positive	32 (66.7)	32 (24.2)[d]	11 (28.2)[c]	43 (25.1)[d]
Tumor differentiation phenotype				
G-phenotype	2 (4.2)	30 (22.7)[d]	10 (25.6)[d]	40 (23.4)[d]
GI-phenotype	19 (39.6)	82 (62.1)[d]	24 (61.5)[d]	106 (62.0)[d]
I-phenotype	27 (56.3)	20 (15.2)[d]	5 (12.8)[d]	25 (14.6)[d]
APC				
Negative	29 (60.4)	110 (83.3)[b]	37 (94.9)[c]	147 (86.0)[d]
Positive	19 (39.6)	22 (16.7)[b]	2 (5.1)[c]	24 (14.0)[d]
p53				
Negative	46 (95.8)	99 (75.0)[b]	27 (69.2)[b]	126 (73.7)[b]
Positive	2 (4.2)	33 (25.0)[b]	12 (30.8)[b]	45 (26.3)[b]
MSI status				
MSS	40 (83.3)	107 (81.1)	34 (87.2)	141 (82.5)
MSI-L	4 (8.3)	16 (12.1)	2 (5.1)	18 (10.5)
MSI-H	4 (8.3)	9 (6.8)	3 (7.7)	12 (7.0)

HGM: Human gastric mucin, [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$, [d]$P < 0.0001$ *vs* adenoma.

tumors (57.1%) and 52 I-phenotype tumors (23.7%). *APC* mutations were detected in 43 (19.6%) of the 219 tumors. Of these mutations, 30 (69.8%) were frameshift mutations (20 deletions and 10 insertions) (Figure 3) and 13 (30%) were point mutations (3 missense and 10 nonsense). *p53* mutations were detected in 47 (21.5%) of the 219 tumors. Of these mutations, 38 (80.9%) were point mutations (35 missense and 3 nonsense) and 9 (19.1%) were frameshift mutations (9 deletions). MSI was observed in 38 (17.4%) of the 219 tumors (Figure 4). MSI-L and MSI-H tumors were observed in 22 (10.0%) and 16 (7.3%) of the 219 tumors, respectively.

Tumor differentiation phenotype and genetic alterations in gastric adenoma and carcinoma

The tumor differentiation phenotype, presence of *APC* or *p53* mutations, and the MSI-status in gastric adenomas, early and advanced carcinomas examined in this study are shown in Table 1. Gastric adenomas were significantly associated with CD10 expression, I-phenotype and *APC* mutations compared with carcinomas (66.7% *vs* 25.1%, $P < 0.0001$; 56.3% *vs* 14.6%, $P < 0.0001$; and 39.6% *vs* 14.0%, $P < 0.0001$, respectively), and inversely associated with HGM and MUC6 expression and *p53* mutations (10.4% *vs* 62.6%, $P < 0.0001$; 39.6% *vs* 64.3%, $P = 0.0030$; 4.2% *vs* 26.3%, $P = 0.0010$, respectively). There were no differences in any of the variables listed in Table 1 between early and advanced carcinomas.

Relations between HGM, MUC6, MUC2, or CD10 expressions and genetic alterations

The relations between the expressions of HGM, MUC6, MUC2, or CD10 and *APC* mutations, *p53* mutations, and the MSI status in gastric tumors are shown in Table 2. The frequency of *APC* mutation was significantly higher in HGM-negative, MUC6-negative and CD10-positive tumors than in HGM-positive, MUC6-positive and CD10-negative tumors (32.7% *vs* 7.1 %, $P < 0.0001$; 27.8% *vs* 14.0%, $P = 0.0182$; and 37.3% *vs* 10.4%, $P = 0.0398$, respectively). The frequency of MSI was significantly higher in MUC6-positive and CD10-negative tumors than in MUC6-negative and CD10-positive tumors (24.8% *vs* 6.7%, $P = 0.0009$ and 22.2% *vs* 8.0%, $P = 0.0143$, respectively). There were no significant differences in the frequency of *p53* mutations according to the expressions of HGM, MUC6, MUC2 or CD10.

Table 2 Relationship between expressions of HGM, MUC6, MUC2 and CD10, and genetic alterations in 219 gastric tumors *n* (%)

	HGM		MUC6		MUC2		CD10	
	Negative (*n* = 107)	Positive (*n* = 112)	Negative (*n* = 90)	Positive (*n* = 129)	Negative (*n* = 56)	Positive (*n* = 163)	Negative (*n* = 144)	Positive (*n* = 75)
APC	35 (32.7)	8 (7.1)[c]	25 (27.8)	18 (14.0)[a]	10 (17.9)	33 (20.2)	15 (10.4)	28 (37.3)[c]
p53	21 (19.6)	26 (23.2)	19 (21.1)	28 (21.7)	10 (17.9)	37 (22.7)	28 (19.4)	19 (25.3)
MSI status								
MSI-L	12 (11.2)	12 (10.7)	4 (4.4)	20 (15.5)	7 (12.5)	17 (10.4)	17 (11.8)	6 (8.0)
MSI-H	7 (6.5)	7 (6.3)	2 (2.2)	12 (9.3)	5 (8.9)	9 (5.5)	15 (10.4)	0 (0)
Total	19 (17.8)	19 (17.0)	6 (6.7)	32 (24.8)[b]	12 (21.4)	26 (16.0)	32 (22.2)	6 (8.0)[a]

HGM: Human gastric mucin, [a]$P < 0.05$, [b]$P < 0.001$, [c]$P < 0.0001$ *vs* negative.

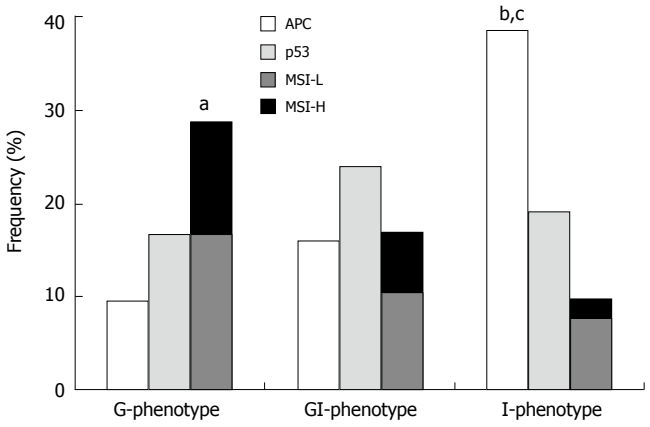

Figure 5 Frequency of *APC*, *p53* mutation and MSI in gastric- (G-), gastric and intestinal mixed- (GI-), and intestinal- (I-) phenotype tumors. [a]$P = 0.0353$ (G-phenotype *vs* I-phenotype, 28.6% *vs* 9.6%), [b]$P = 0.0017$ (I-phenotype *vs* G-phenotype, 38.5% *vs* 9.5%), [c]$P = 0.0022$ (I-phenotype *vs* GI-phenotype, 38.5% *vs* 16.0%).

Relations between tumor differentiation phenotype and genetic alterations

The relations between tumor differentiation phenotype and *APC* mutations, *p53* mutations and the MSI-status in gastric tumors are shown in Figure 5. The frequency of *APC* mutations was significantly higher in I-phenotype tumors than in G- phenotype and GI-phenotype tumors (38.5% *vs* 9.5% and 16.0%, $P = 0.0017$ and $P = 0.0022$, respectively). The frequency of MSI was significantly higher in G-phenotype tumors than in I-phenotype tumors (28.6% *vs* 9.6%, $P = 0.0353$). There were no significant differences in the frequency of *p53* mutations among the G-, GI- and I-phenotypes.

DISCUSSION

Our present results revealed that gastric adenomas were significantly associated with CD10 expression, I-phenotype and *APC* mutation and inversely associated with HGM and MUC6 expression and *p53* mutations, compared with early and advanced carcinomas. Gastric adenomas were also significantly associated with MUC2 expression, compared with advanced carcinomas. Therefore, gastric adenomas are clearly different from early and advanced carcinomas in terms of the tumor differentiation phenotype and genetic alterations.

With respect to the difference in the tumor differentiation phenotype between gastric adenomas and carcinomas, our present results revealed that more than half of gastric adenomas exhibited I-phenotype and the incidence of I-phenotype tumor was significantly higher in gastric adenomas than in early and advanced carcinomas. On the other hand, the majority of early and advanced carcinomas exhibited a G- or GI-phenotype. These findings indicate that the tumor differentiation phenotype is related to tumor invasion in gastric differentiated-type tumors. Recently, several reports have demonstrated distinct differences in the biological behavior of tumors with different phenotypic markers[8-10,14-18,26]. Endoh *et al*[34] reported that low positivity for Ki-67 staining along the mature collagenous stromal reaction in the invasive area can be seen in differentiated-type carcinomas of I-phenotype, reflecting the slow growth of this tumor. We have previously reported that patients with G-phenotype tumors have a significantly poorer prognosis than those with I-phenotype tumors in patients with advanced gastric carcinoma[8].

In the present study, gastric adenomas were significantly associated with *APC* mutations and inversely associated with *p53* mutations, compared with those in early and advanced carcinomas. MSI is a common event in both gastric adenomas and differentiated-type carcinomas. The absence of sequential accumulations of genetic alterations between gastric adenomas and early or advanced carcinomas suggests that gastric differentiated-type tumors with *APC* mutations, especially gastric adenomas, may have a low invasive potential. Lee *et al*[31] suggested that gastric adenomas without *APC* mutations have a different biological behavior from those with *APC* mutations and are precursors of differentiated-type carcinoma. Distinct differences in the tumor differentiation phenotype and genetic alterations between gastric adenomas and differentiated-type carcinomas suggest that the adenoma-carcinoma sequence, typically seen in colorectal carcinoma, is not be a major pathway in tumorigenesis of gastric differentiated-type tumor. Furthermore, our present findings also suggest that the tumor differentiation phenotype and genetic alterations are related to tumor aggressiveness in gastric differentiated-type tumors.

The relations between tumor differentiation phenotype and genetic alterations have not been examined in any large study on gastric adenoma and early differentiated-type carcinoma. Our present results revealed that MSI was

significantly associated with MUC6 expression and G-phenotype, and inversely associated with CD10 expression. On the other hand, *APC* mutation was significantly associated with CD10 expression and I-phenotype, and inversely associated with expressions of HGM and MUC6, suggesting that the tumor differentiation phenotype is closely related to genetic alterations in gastric differentiated-type tumors. Differentiation of gastric epithelial cells, especially pyloric gland cells, may be related to MSI, while differentiation of intestinal epithelial cells, especially absorptive cells, may be related to *APC* mutations. To the best of our knowledge, this is the first report describing associations between genetic alterations, such as *APC* mutations and MSI, and the expressions of HGM, MUC6, MUC2 or CD10 in gastric differentiated-type tumors.

The differences in the biological behavior of tumors with different phenotype as described above suggest that the action of different genetic alterations depends on the tumor differentiation phenotype of gastric tumors. Kabashima *et al*[10] reported that G-phenotype tumors can potentially degrade the extracelluar matrix through the overexpression of matrix metalloproteinases, compared with I-phenotype tumors. Shibata *et al*[17] reported that the apototic index/proliferative index ratio is significantly lower in G-phenotype tumors than in I-phenotype tumors. Endoh *et al*[18] have detected *E-cadherin* mutations in 21% of differentiated-type carcinomas with a G-phenotype, although this mutation is generally considered to be involved in undifferentiated-type but not differentiated-type carcinomas. Our present findings show that the tumor differentiation phenotype is closely related to tumor invasion and genetic alterations in gastric differentiated-type tumors. Previous molecular genetics studies have shown that gastric tumorigenesis is a multistep process with accumulation of genetic alterations[3,4]. Therefore, the previous data and our present findings suggest that different genetic pathways according to the tumor differentiation phenotype might play a role in the tumorigenesis of the gastric differentiated-type tumor, leading to their different biological behavior.

In conclusion, the tumor differentiation phenotype is closely related to tumor invasion and genetic alterations in gastric differentiated-type tumors.

REFERENCES

1 **LAUREN P**. THE TWO HISTOLOGICAL MAIN TYPES OF GASTRIC CARCINOMA: DIFFUSE AND SO-CALLED INTESTINAL-TYPE CARCINOMA. AN ATTEMPT AT A HISTO-CLINICAL CLASSIFICATION. *Acta Pathol Microbiol Scand* 1965; **64**: 31-49

2 **Nakamura K**, Sugano H, Takagi K. Carcinoma of the stomach in incipient phase: its histogenesis and histological appearances. *Gann* 1968; **59**: 251-258

3 **Stadtländer CT**, Waterbor JW. Molecular epidemiology, pathogenesis and prevention of gastric cancer. *Carcinogenesis* 1999; **20**: 2195-2208

4 **Tahara E**, Semba S, Tahara H. Molecular biological observations in gastric cancer. *Semin Oncol* 1996; **23**: 307-315

5 **Nakatsuru S**, Yanagisawa A, Furukawa Y, Ichii S, Kato Y, Nakamura Y, Horii A. Somatic mutations of the APC gene in precancerous lesion of the stomach. *Hum Mol Genet* 1993; **2**: 1463-1465

6 **Chong JM**, Fukayama M, Hayashi Y, Takizawa T, Koike M, Konishi M, Kikuchi-Yanoshita R, Miyaki M. Microsatellite instability in the progression of gastric carcinoma. *Cancer Res* 1994; **54**: 4595-4597

7 **Hiyama E**, Yokoyama T, Tatsumoto N, Hiyama K, Imamura Y, Murakami Y, Kodama T, Piatyszek MA, Shay JW, Matsuura Y. Telomerase activity in gastric cancer. *Cancer Res* 1995; **55**: 3258-3262

8 **Tajima Y**, Shimoda T, Nakanishi Y, Yokoyama N, Tanaka T, Shimizu K, Saito T, Kawamura M, Kusano M, Kumagai K. Gastric and intestinal phenotypic marker expression in gastric carcinomas and its prognostic significance: immunohistochemical analysis of 136 lesions. *Oncology* 2001; **61**: 212-220

9 **Tajima Y**, Yamazaki K, Nishino N, Morohara K, Yamazaki T, Kaetsu T, Suzuki S, Kawamura M, Kumagai K, Kusano M. Gastric and intestinal phenotypic marker expression in gastric carcinomas and recurrence pattern after surgery-immunohistochemical analysis of 213 lesions. *Br J Cancer* 2004; **91**: 1342-1348

10 **Kabashima A**, Yao T, Sugimachi K, Tsuneyoshi M. Relationship between biologic behavior and phenotypic expression in intramucosal gastric carcinomas. *Hum Pathol* 2002; **33**: 80-86

11 **Tatematsu M**, Ichinose M, Miki K, Hasegawa R, Kato T, Ito N. Gastric and intestinal phenotypic expression of human stomach cancers as revealed by pepsinogen immunohistochemistry and mucin histochemistry. *Acta Pathol Jpn* 1990; **40**: 494-504

12 **Egashira Y**, Shimoda T, Ikegami M. Mucin histochemical analysis of minute gastric differentiated adenocarcinoma. *Pathol Int* 1999; **49**: 55-61

13 **Sasaki I**, Yao T, Nawata H, Tsuneyoshi M. Minute gastric carcinoma of differentiated type with special reference to the significance of intestinal metaplasia, proliferative zone, and p53 protein during tumor development. *Cancer* 1999; **85**: 1719-1729

14 **Endoh Y**, Tamura G, Watanabe H, Ajioka Y, Motoyama T. The common 18-base pair deletion at codons 418-423 of the E-cadherin gene in differentiated-type adenocarcinomas and intramucosal precancerous lesions of the stomach with the features of gastric foveolar epithelium. *J Pathol* 1999; **189**: 201-206

15 **Koseki K**, Takizawa T, Koike M, Ito M, Nihei Z, Sugihara K. Distinction of differentiated type early gastric carcinoma with gastric type mucin expression. *Cancer* 2000; **89**: 724-732

16 **Saito A**, Shimoda T, Nakanishi Y, Ochiai A, Toda G. Histologic heterogeneity and mucin phenotypic expression in early gastric cancer. *Pathol Int* 2001; **51**: 165-171

17 **Shibata N**, Watari J, Fujiya M, Tanno S, Saitoh Y, Kohgo Y. Cell kinetics and genetic instabilities in differentiated type early gastric cancers with different mucin phenotype. *Hum Pathol* 2003; **34**: 32-40

18 **Endoh Y**, Sakata K, Tamura G, Ohmura K, Ajioka Y, Watanabe H, Motoyama T. Cellular phenotypes of differentiated-type adenocarcinomas and precancerous lesions of the stomach are dependent on the genetic pathways. *J Pathol* 2000; **191**: 257-263

19 **Schlemper RJ**, Riddell RH, Kato Y, Borchard F, Cooper HS, Dawsey SM, Dixon MF, Fenoglio-Preiser CM, Fléjou JF, Geboes K, Hattori T, Hirota T, Itabashi M, Iwafuchi M, Iwashita A, Kim YI, Kirchner T, Klimpfinger M, Koike M, Lauwers GY, Lewin KJ, Oberhuber G, Offner F, Price AB, Rubio CA, Shimizu M, Shimoda T, Sipponen P, Solcia E, Stolte M, Watanabe H, Yamabe H. The Vienna classification of gastrointestinal epithelial neoplasia. *Gut* 2000; **47**: 251-255

20 **Bara J**, Chastre E, Mahiou J, Singh RL, Forgue-Lafitte ME, Hollande E, Godeau F. Gastric M1 mucin, an early oncofetal marker of colon carcinogenesis, is encoded by the MUC5AC gene. *Int J Cancer* 1998; **75**: 767-773

21 **De Bolós C**, Garrido M, Real FX. MUC6 apomucin shows a distinct normal tissue distribution that correlates with Lewis antigen expression in the human stomach. *Gastroenterology* 1995; **109**: 723-734

22 **Machado JC**, Nogueira AM, Carneiro F, Reis CA, Sobrinho-Simões M. Gastric carcinoma exhibits distinct types of cell differentiation: an immunohistochemical study of trefoil peptides (TFF1 and TFF2) and mucins (MUC1, MUC2, MUC5AC, and MUC6). *J Pathol* 2000; **190**: 437-443

23 **Kim YS**, Gum JR Jr. Diversity of mucin genes, structure, func-

tion, and expression. *Gastroenterology* 1995; **109**: 999-1001
24 **Ronco P**, Allegri L, Melcion C, Pirotsky E, Appay MD, Bariety J, Pontillon F, Verroust P. A monoclonal antibody to brush border and passive Heymann nephritis. *Clin Exp Immunol* 1984; **55**: 319-332
25 **Hsu SM**, Raine L, Fanger H. Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem* 1981; **29**: 577-580
26 **Tsukashita S**, Kushima R, Bamba M, Sugihara H, Hattori T. MUC gene expression and histogenesis of adenocarcinoma of the stomach. *Int J Cancer* 2001; **94**: 166-170
27 **Konishi K**, Yamochi T, Makino R, Kaneko K, Yamamoto T, Nozawa H, Katagiri A, Ito H, Nakayama K, Ota H, Mitamura K, Imawari M. Molecular differences between sporadic serrated and conventional colorectal adenomas. *Clin Cancer Res* 2004; **10**: 3082-3090
28 **Makino R**, Kaneko K, Kurahashi T, Matsumura T, Mitamura K. Detection of mutation of the p53 gene with high sensitivity by fluorescence-based PCR-SSCP analysis using low-pH buffer and an automated DNA sequencer in a large number of DNA samples. *Mutat Res* 2000; **452**: 83-90
29 **Baba S**, Kukita Y, Higasa K, Tahira T, Hayashi K. Single-stranded conformational polymorphism analysis using automated capillary array electrophoresis apparatuses. *Biotechniques* 2003; **34**: 746-750
30 **Higasa K**, Kukita Y, Baba S, Hayashi K. Software for machine-independent quantitative interpretation of SSCP in capillary array electrophoresis (QUISCA). *Biotechniques* 2002; **33**: 1342-1348
31 **Lee JH**, Abraham SC, Kim HS, Nam JH, Choi C, Lee MC, Park CS, Juhng SW, Rashid A, Hamilton SR, Wu TT. Inverse relationship between APC gene mutation in gastric adenomas and development of adenocarcinoma. *Am J Pathol* 2002; **161**: 611-618
32 **Boland CR**, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 1998; **58**: 5248-5257
33 **Kolodziejczyk P**, Yao T, Oya M, Nakamura S, Utsunomiya T, Ishikawa T, Tsuneyoshi M. Long-term follow-up study of patients with gastric adenomas with malignant transformation. An immunohistochemical and histochemical analysis. *Cancer* 1994; **74**: 2896-2907
34 **Endoh Y**, Tamura G, Motoyama T, Ajioka Y, Watanabe H. Well-differentiated adenocarcinoma mimicking complete-type intestinal metaplasia in the stomach. *Hum Pathol* 1999; **30**: 826-832

S- Editor Pan BR **L- Editor** Wang XL **E- Editor** Ma WH