Gastric and Intestinal Phenotypic Marker Expression in Early Differentiated-Type Tumors of the Stomach: Clinicopathologic Significance and Genetic Background

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Abstract

Purpose: Gastric and intestinal phenotypic cell markers are expressed in gastric carcinomas, irrespective of their histologic type. In the present study, we determined the clinicopathologic significance of phenotypic marker expression in early-stage gastric differentiated-type tumors and the association between marker expression and genetic alterations.

Experimental Design: Phenotypic marker expression was determined by examining the expressions of human gastric mucin (HGM), MUC6, MUC2, and CD10 in 63 gastric adenomas, 133 early differentiated-type carcinomas, and 24 follow-up cases with gastric adenoma. Tumors were classified into gastric, gastric and intestinal mixed, or intestinal phenotypes according to the immunopositivity of the above markers. The presence of mutations in *APC, K-ras*, and *p53* and the microsatellite instability status were also determined in all tumors.

Results: The expressions of HGM and MUC6, representing gastric or gastric and intestinal mixed phenotypes, were significantly associated with high-grade atypia in the 63 gastric adenomas. Among the 133 early differentiated-type carcinomas, HGM expression was significantly associated with mixed-type (with an undifferentiated-type component) tumors and lymph node metastasis. MUC2 expression was inversely associated with submucosal invasion. A multivariate analysis revealed that gastric adenomas were significantly associated with the intestinal phenotype and were inversely associated with *p53* mutation compared with early differentiated-type carcinomas. Among all 196 tumors, *APC* mutation was significantly associated with CD10 expression and the intestinal phenotype and was inversely associated with the expressions of HGM and MUC6. The microsatellite instability status was significantly associated with MUC6 expression. Malignant transformation from gastric adenoma to carcinoma was shown in 5 of the 24 follow-up cases of gastric adenoma. The malignant transformation was significantly associated with the gastric and intestinal mixed phenotype and was inversely associated with *APC* mutation. No malignant transformation was found in intestinal phenotype gastric adenomas with *APC* mutation.

Conclusions: Our present findings show that phenotypic marker expression is associated with tumor aggressiveness during the early stage of gastric differentiated-type tumors. Differences in the biological behavior of tumors with different phenotypes may result from differences in the genetic backgrounds during the incipient phase of gastric tumorigenesis.

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Gastric carcinoma is histologically classified into two types, differentiated and undifferentiated or intestinal and diffuse type, based on the gland formation tendency (1, 2). With respect to the histogenesis of these two types of gastric carcinoma, differentiated-type tumors have generally been considered to arise from gastric mucosa with intestinal metaplasia and undifferentiated-type tumors from ordinary gastric mucosa without intestinal metaplasia; these two tumor types are believed to follow different genetic pathways during carcinogenesis (3). However, both gastric and intestinal phenotypic cell markers are expressed in gastric carcinomas, irrespective of their histologic type. We and other investigators have reported that gastric carcinomas are classified as having either a gastric, gastric

Table 1. Literature series of phenotypic marker expression in gastric tumors

Author, y	No. tumors analyzed	Clinicopathologic findings correlated with phenotypic marker expression	Other findings correlated with phenotypic marker expression
Yamachika (18), 1997	203 signet ring cell carcinomas	Tumor intestinalization	—
Yoshikawa (19), 1998	301 differentiated-type carcinomas	Tumor intestinalization	—
Endoh (10), 1999	10 differentiated-type carcinomas 9 precancerous lesions	—	*E-cadherin* mutation —
Endoh (11), 2000	differentiated-type carcinomas precancerous lesions (52 cases)	—	*p53* mutation MSI
Koseki (12), 2000	120 submucosal carcinomas	Papillary-type carcinoma Lymph node metastasis	E-cadherin expression
Saito (7), 2001	351 early carcinomas	Histologic transformation	—
Tajima (13), 2001	136 advanced carcinomas	Patients prognosis	—
Tsukashita (14), 2001	80 intramucosal neoplastic lesions	Grade of atypia	—
Kabashima (8), 2002	114 intramucosal carcinomas	—	Matrix metalloproteinase-9
Shibata (9), 2003	43 early carcinomas	—	Apoptotic index/proliferative index
Tajima (15), 2003	137 advanced carcinomas	Chemosensitivity	Thymidylate synthase expression
Tajima (16), 2004	213 advanced carcinomas	Recurrence pattern	—
Yamazaki (17), 2006	48 adenomas, 171 carcinomas	Tumor invasion	*APC* mutation, MSI
Morohara (20), 2006	34 advanced carcinomas	—	Chromosomal changes, E-cadherin expression
This series	63 adenomas, 133 early carcinomas, 24 follow-up cases with adenoma	Histologic type Histologic transformation Submucosal invasion Lymph node metastasis	*APC* mutation *p53* mutation MSI

and intestinal mixed, or intestinal phenotype depending on the expressions of human gastric mucin (HGM; a marker of the gastric foveolar epithelium), MUC6 (a marker of gastric pyloric gland cells), MUC2 (a marker of intestinal goblet cells), and CD10 (a marker of intestinal absorptive cells; refs. 4–20). Gastric phenotype tumors account for 27.7% of differentiated tumors, often referred to as intestinal-type tumors according to Lauren (2), whereas intestinal phenotype tumors accounted for 10.1% of undifferentiated tumors (13). The phenotypic marker expression of tumors is conventionally thought to imitate that of the tissue of origin. Thus, the above data suggest that gastric carcinomas can occur in various types of gastric mucosa.

Recently, the phenotypic marker expression of tumors has also been associated with tumor aggressiveness in gastric carcinomas (Table 1). We previously reported that patients with gastric phenotype tumors were significantly associated with a high risk of peritoneal recurrence and a poorer outcome after surgical resection compared with those with intestinal phenotype tumors among patients with advanced gastric carcinoma (13, 16). We also previously reported that the intratumoral expression of thymidylate synthase, the target enzyme of 5-fluorouracil, was significantly low in gastric phenotype tumors and that postoperative chemotherapy with 5-fluorouracil was effective for patients with gastric phenotype tumors (15). Therefore, phenotypic marker expression is closely related to both gastric tumorigenesis and the biological behavior of gastric carcinomas. Furthermore, appropriate therapeutic approaches for patients with gastric carcinomas might differ according to the phenotypic marker expression of the tumor.

The phenotypic marker expression in gastric carcinomas has been suggested to be dependent on genetic changes (10, 11, 20). Previous molecular genetic studies have shown that gastric tumorigenesis is a multistep process involving the accumulation of genetic alterations (3, 21). Therefore, to clarify gastric tumorigenesis, it is essential to investigate phenotypic marker expression and genetic alterations in early gastric tumors. However, the association between the phenotypic marker expression and genetic alterations in gastric adenomas and early differentiated-type carcinomas remains unclear (Table 1). Even early-stage gastric differentiated-type tumors may have different characteristics in terms of biological behavior and related genetic alterations according to the phenotypic marker expression of the tumors.

In the present study, the phenotypic marker expressions and related genetic alterations, such as mutations in *APC*, *K-ras*, and *p53* and the microsatellite instability (MSI) status, were examined in 63 gastric adenomas, 133 early differentiated-type carcinomas, and 24 follow-up cases with gastric adenoma. The

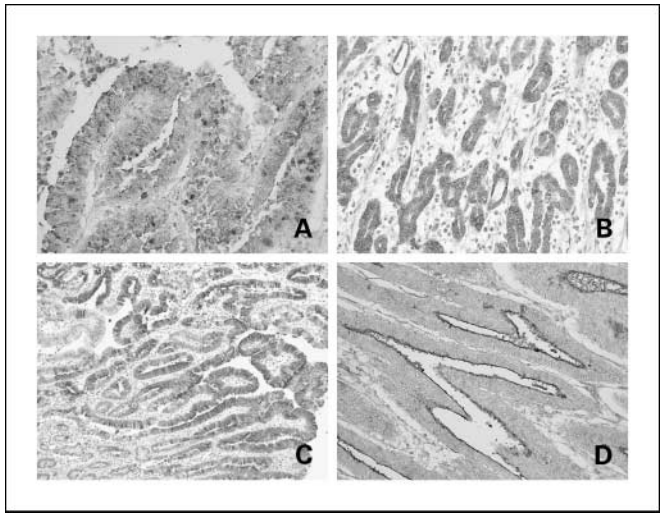

Fig. 1. Immunohistochemical analysis of phenotypic marker expressions in gastric tumor. *A,* HGM is expressed in the cancer cell cytoplasm (45M1). Original magnification, ×200. *B,* MUC6 glycoprotein is expressed in the cancer cell cytoplasm (CLH5). Original magnification, ×100. *C,* MUC2 glycoprotein is expressed in the cancer cell cytoplasm (Ccp58). Original magnification, ×100. *D,* CD10 glycoprotein is expressed on the luminal surfaces of cancerous glands (56C6). Original magnification, ×200.

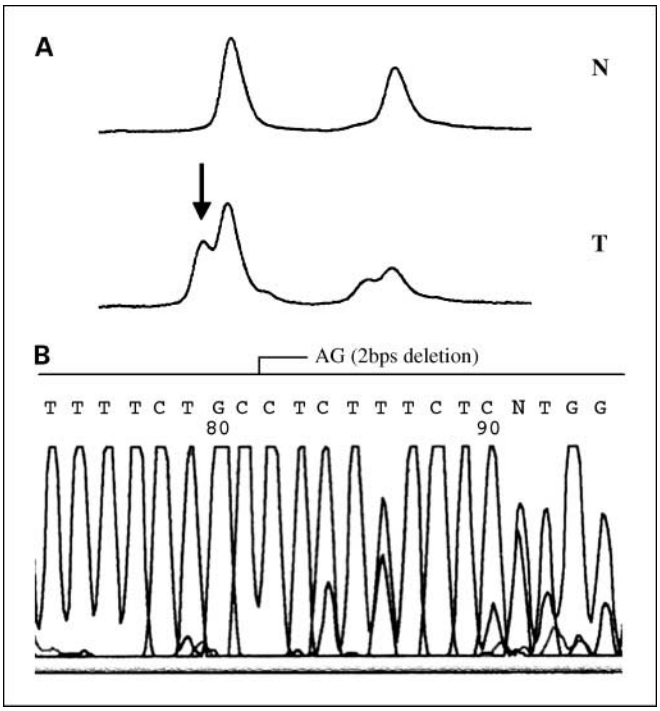

Fig. 2. Mutation analysis of *APC* in gastric tumor. *A,* PCR single-strand conformation polymorphism analysis shows shift peak (*arrowhead*) compared with the control normal DNA. *B,* DNA sequence analysis reveals frameshift mutation (AG, 2 bp deletion) at codon 1,511 of *APC*. T, tumor DNA; N, normal DNA.

purpose of the present study was to clarify the clinicopathologic significance of the phenotypic marker expression of gastric adenomas and early differentiated-type carcinomas and the genetic background during the incipient phase of gastric tumorigenesis.

Materials and Methods

Patients. Our series consisted of 63 patients with gastric adenoma who had undergone endoscopic mucosal resections or biopsies, 133 patients with early differentiated-type adenocarcinoma who had undergone surgical resections, and 24 follow-up patients who had undergone endoscopic mucosal resections or biopsies between 2001 and 2003 at Showa University Hospital (Tokyo, Japan). Written informed consent was obtained from all the patients before they were included in the present study.

Histologic review. The specimens were fixed with 10% buffered formalin and embedded in paraffin. The 4-μm consecutive sections were used for histopathologic examination by H&E staining and immunohistochemical stainings. The intramucosal neoplastic lesions were classified histologically according to the general rules established by the Vienna classification (22).

Analysis of phenotypic marker expression. The following mouse monoclonal antibodies were used: 45M1, diluted 1:50, to detect HGM; CLH5, diluted 1:50, to detect MUC6 glycoprotein; Ccp58, diluted 1:100, to detect MUC2 glycoprotein; and 56C6, diluted 1:40, to detect CD10 glycoprotein expression (all from Novocastra Laboratories Ltd., Newcastle upon Tyne, United Kingdom). 45M1 and CLH5 were examined as gastric phenotype markers and Ccp58 and 56C6 were examined as intestinal phenotype markers. HGM is synonymous with MUC5AC, and the antibody is known to react with surface foveolar cells in the stomach (23, 24). MUC6 glycoprotein is expressed in mucous cells of the neck zone of oxyntic mucosa and in antral glands (25, 26). MUC2 glycoprotein is an intestinal apomucin and is also expressed in the supranuclear area of the goblet cells in regions showing intestinal metaplasia in the stomach (26, 27). CD10 glycoprotein is expressed on the brush border of intestinal epithelial cells (28, 29). The avidin-biotinyl-peroxidase complex immunohistochemical method was used for all immunohistochemical studies according to a previously described protocol (30).

With regard to the evaluations of HGM, MUC6, MUC2, and CD10 staining, distinct staining in >5% of the tumor cells was recorded as positive immunoreactivity for the relevant marker (Fig. 1). These immunohistochemical methods were used to classify the tumors into three different phenotypes: tumors with gastric phenotypic cells accounting for >5% of their cell population were classified as gastric phenotype tumors; those with intestinal phenotypic cells accounting for >5% of their cell population were classified as intestinal phenotype tumors; and those with both gastric and intestinal phenotypic cells accounting for >5% of their cell population were classified as gastric and intestinal mixed phenotype tumors (14, 16, 20).

DNA extraction. Microdissection from 10-μm-thick formalin-fixed, paraffin-embedded serial section was done on H&E-stained slides for both tumor and normal mucosa. Tissues were precisely microdissected under microscopic visualization using a PixCell laser capture microdissection system (Arcus Engineering, Mountain View, CA) to avoid the DNA contamination of inflammatory or stromal cell nuclei and DNA of cancerous cells without overt differentiation phenotype of the tumor. Genomic DNA was extracted from microdissected tissue as described previously (31).

Gene mutation analysis. Mutations of *APC* in exon 15, codon 1,260 to 1,596 region, and *K-ras* in codon 12 to 13 regions were detected by fluorescence-based PCR single-strand conformation polymorphism analysis with previously described primers (17, 32, 33). After amplification, the PCR products from each sample were used to detect *APC* or *K-ras* mutations by fluorescence-based single-strand conformation polymorphism analysis using the ALFexpress DNA sequencer (Amersham Biosciences, Piscataway, NJ) with a cooling bath. Peak patterns were analyzed with the ALFwin Fragment Analyzer Program (Amersham Biosciences), and shift peaks were defined as mutations in DNA fragments. The nucleotide sequences of the DNA fragments with shifted peaks were previously determined. All mutations were reconfirmed by independent PCRs and sequencing.

Mutations of *p53* in the exons 5 to 8 region were detected by fluorescence-based PCR single-strand conformation polymorphism using capillary electrophoresis (17). The nucleotide sequences of the primers were described previously. Mutations were detected by electrophoresis with an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). The peak pattern was analyzed with QUISCA software, established by Higasa et al. and kindly provided by

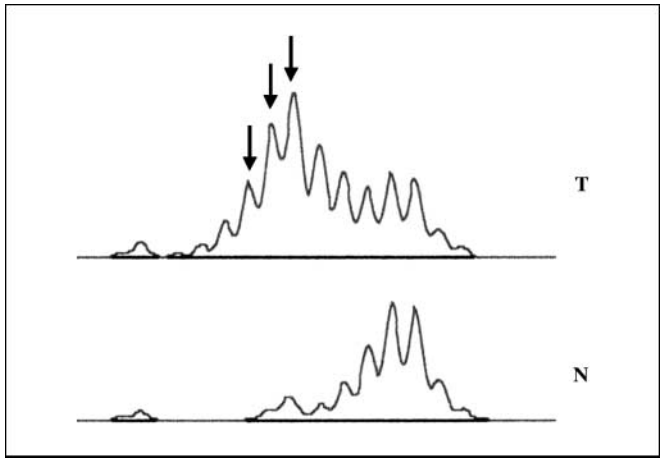

Fig. 3. MSI analysis in gastric tumor. MSI analysis using marker BAT-26 shows a different allelic shift peak (*arrowhead*) compared with the control normal DNA.

Dr. K. Hayashi (Division of Genome Analysis, Research Center for Genetic Information, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan) (17, 31, 34, 35). The nucleotide sequences of DNA fragments with shifted peaks were determined using a Genetic Analyzer 310 with a BigDye Terminator (Applied Biosystems). All mutations were reconfirmed by independent PCRs and sequencing (Fig. 2).

MSI analysis. MSI was detected with five microsatellite markers: BAT-25, BAT-26, D2S123, D5S346, and D17S250. The primers and PCR conditions have been described elsewhere (17, 31). 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 DNA that was not present in the control DNA. A specimen was considered to be MSI positive when at least one marker showed an allelic shift. A tumor sample was considered to contain high-frequency MSI (MSI-H) if two or more of the five informative markers showed instability and was considered to have low-frequency MSI (MSI-L) when only one marker was unstable. All PCRs were repeated on the same sample, and only consistent changes in the duplicate reactions were scored as abnormalities (Fig. 3).

Statistical analysis. Statistical comparisons were done with the Student's t test or the Mann-Whitney U test between two independent groups and with the χ^2 test or the Fisher's exact test between two proportions. Univariate and multivariate analyses to assess differences in the clinicopathologic findings, phenotypic marker expressions, and genetic alterations between gastric adenomas and early differentiated-type carcinomas were done using logistic regression analysis. The level of significance was set at $P < 0.05$.

Results

Phenotypic marker expressions and genetic alterations in 63 gastric adenomas and 133 early differentiated-type carcinomas. The expression of HGM, MUC6, MUC2, and CD10 was shown

Table 2. Relationships between clinicopathologic findings and phenotypic marker expression in early gastric tumors

A. Relationships between clinicopathologic findings and phenotypic marker expressions in 63 gastric adenomas

	HGM expression		MUC6 expression		MUC2 expression		CD10 expression		PMEP		
	Negative (n = 57)	Positive (n = 6)	Negative (n = 41)	Positive (n = 22)	Negative (n = 7)	Positive (n = 56)	Negative (n = 22)	Positive (n = 41)	G (n = 2)	GI (n = 23)	I (n = 38)
Macroscopic type											
Elevated	55 (96.5)	4 (66.7)	39 (95.1)	20 (90.9)	6 (85.7)	53 (94.6)	20 (90.9)	39 (95.1)	2 (100)	21 (91.3)	36 (94.7)
Flat, depressed	2 (3.5)	2 (33.3)	2 (9.1)	2 (9.1)	1 (14.3)	3 (5.4)	2 (9.1)	2 (4.9)	0 (0)	2 (8.7)	2 (5.3)
Tumor size											
Mean (mm)	9.3	10.8	8.0	11.5	8.3	9.6	9.8	9.2	98.0	11.3	8.2
Histologic type*											
Low grade	46 (80.7)	2 (33.3)	36 (87.8)	12 (54.5)	4 (57.1)	44 (78.6)	15 (68.2)	33 (80.5)	1 (50.0)	14 (60.9)	33 (86.8)
High grade	11 (19.3)	4 (66.7)	5 (12.2)	10 (45.5)	3 (42.9)	12 (21.4)	7 (31.8)	8 (19.5)	1 (50.0)	9 (39.1)	5 (13.2)

B. Relationships between clinicopathologic findings and phenotypic marker expressions in 63 gastric adenomas

	HGM expression		MUC6 expression		MUC2 expression		CD10 expression		PMEP		
	Negative (n = 48)	Positive (n = 85)	Negative (n = 48)	Positive (n = 85)	Negative (n = 35)	Positive (n = 98)	Negative (n = 100)	Positive (n = 33)	G (n = 30)	GI (n = 83)	I (n = 20)
Macroscopic type											
Elevated	19 (39.6)	29 (34.1)	17 (35.4)	31 (36.5)	10 (28.6)	38 (38.8)	38 (38.0)	10 (30.3)	9 (30.0)	31 (37.3)	8 (40.0)
Flat, depressed	29 (60.4)	56 (65.9)	31 (64.6)	54 (63.5)	25 (71.4)	60 (61.2)	62 (62.0)	23 (69.7)	21 (70.0)	52 (62.7)	12 (60.0)
Tumor size											
Mean (mm)	26.2	27.1	26.8	26.9	27	26.2	27.6	23.0	29.7	25.4	27.0
Histologic type†											
Pure differentiated	48 (100)	75 (88.2)	45 (93.8)	78 (91.8)	31 (88.6)	92 (93.9)	91 (91.0)	32 (97.0)	26 (86.7)	77 (92.8)	20 (100)
Mixed with undifferentiated	0 (0)	10 (11.8)	3 (6.3)	7 (8.1)	4 (11.4)	6 (6.1)	9 (9.0)	1 (3.0)	4 (13.3)	6 (7.2)	0 (0)
Depth of invasion‡											
M	34 (70.8)	46 (54.1)	25 (52.1)	55 (64.7)	15 (42.9)	65 (66.3)	57 (57.0)	23 (69.7)	14 (46.7)	52 (62.7)	14 (70.0)
SM	14 (29.2)	39 (45.9)	23 (47.9)	30 (35.3)	20 (57.1)	33 (33.7)	43 (43.0)	10 (30.3)	16 (53.3)	31 (37.3)	6 (30.0)
Lymphatic permeation											
Negative	42 (87.5)	66 (77.6)	39 (81.3)	69 (81.2)	27 (77.1)	81 (82.7)	77 (77.0)	31 (93.9)	24 (80.0)	67 (80.7)	17 (85.0)
Positive	6 (12.5)	19 (22.4)	9 (18.8)	16 (18.8)	8 (22.9)	17 (17.3)	23 (23.0)	2 (6.1)	6 (20.0)	16 (19.3)	3 (15.0)
Blood vessel permeation											
Negative	43 (89.6)	70 (82.4)	39 (81.3)	74 (87.1)	30 (85.7)	83 (84.7)	83 (83.0)	30 (90.9)	24 (80.0)	71 (85.5)	18 (90.0)
Positive	5 (10.4)	15 (17.6)	9 (18.8)	11 (12.9)	5 (14.3)	15 (15.3)	17 (17.0)	3 (9.1)	6 (20.0)	12 (14.4)	2 (10.0)
Lymph node metastasis§											
Negative	48 (100)	78 (91.8)	46 (95.8)	80 (94.1)	33 (94.3)	93 (94.9)	94 (94.0)	32 (97.0)	29 (96.7)	77 (92.8)	20 (100)
Positive	0 (0)	7 (8.2)	2 (4.2)	5 (5.9)	2 (5.7)	5 (5.1)	6 (6.0)	1 (3.0)	1 (3.3)	6 (7.2)	0 (0)

Abbreviations: PMEP, phenotypic marker expression pattern; G, gastric phenotype; GI, gastric and intestinal mixed phenotype; I, intestinal phenotype; M, mucosa; SM, submucosa.
*P = 0.0249 (HGM expression), 0.0051 (MUC6 expression), and 0.0319 (PMEPT).
†P = 0.0137 (HGM expression).
‡P = 0.0149 (MUC2 expression).
§P = 0.0487 (HGM expression).

Table 3. Differences in clinicopathologic findings, phenotypic marker expressions, and genetic alterations between 63 gastric adenomas and 133 early differentiated-type carcinomas

	Adenoma			Early carcinoma			Univariate analysis	Multivariate analysis A		Multivariate analysis B	
	LG (n = 48)	HG (n = 15)	Total (n = 63)	MC (n = 80)	SMC (n = 53)	Total (n = 133)	P	OR (95% CI)	P	OR (95% CI)	P
Macroscopic type											
Elevated	48 (100)	11 (73.3)	59 (93.7)	31 (38.8)	17 (32.1)	48 (36.1)	<0.0001	34.3 (5.5-213.4)	0.0001	36.5 (8.1-164.4)	<0.0001
Flat, depressed	0 (0)	4 (26.7)	4 (6.3)	49 (61.3)	36 (67.9)	85 (63.9)					
Tumor size											
Mean (mm)	8.9	11.2	9.4	24.5	27.8	26.5	<0.0001	1.2 (1.1-1.3)	<0.0001	1.2 (1.1-1.3)	<0.0001
HGM expression											
Negative	46 (95.8)	11 (73.3)	57 (90.5)	34 (42.5)	14 (26.4)	48 (36.1)	<0.0001	25.3 (4.1-155.8)	0.0005		NA
Positive	2 (4.2)	4 (26.7)	6 (9.5)	46 (57.5)	39 (73.6)	85 (63.9)					
MUC6 expression											
Negative	36 (75.0)	5 (33.3)	41 (65.1)	25 (31.3)	23 (43.4)	48 (36.1)	0.0002		NS		NA
Positive	12 (25.0)	10 (66.7)	22 (34.9)	55 (68.8)	30 (56.6)	85 (63.9)					
MUC2 expression											
Negative	4 (8.3)	3 (20.0)	7 (11.1)	15 (18.8)	20 (37.7)	35 (26.3)	0.0187		NS		NA
Positive	44 (91.7)	12 (80.0)	56 (88.9)	65 (81.3)	33 (62.3)	98 (73.7)					
CD10 expression											
Negative	15 (31.3)	7 (46.7)	22 (34.9)	57 (71.3)	43 (81.1)	100 (75.2)	<0.0001		NS		NA
Positive	33 (68.8)	8 (53.3)	41 (65.1)	23 (28.8)	10 (18.9)	33 (24.8)					
PMEP											
G phenotype	1 (2.1)	1 (6.7)	2 (3.2)	14 (17.5)	16 (30.2)	30 (22.6)	<0.0001		NA	5.1 (1.7-15.5)	0.0044
GI phenotype	14 (29.2)	9 (60.0)	23 (36.5)	52 (65.0)	31 (58.5)	83 (62.4)					
I phenotype	33 (68.8)	5 (33.3)	38 (60.3)	14 (17.5)	6 (11.3)	20 (15.0)					
APC mutation											
Negative	25 (52.1)	13 (86.7)	38 (60.3)	64 (80.0)	47 (88.7)	111 (83.5)	0.0006		NS		NS
Positive	23 (47.9)	2 (13.3)	25 (39.7)	16 (20.0)	6 (11.3)	22 (16.5)					
K-ras mutation											
Negative	48 (100)	15 (100)	63 (100)	75 (93.8)	51 (96.2)	126 (94.7)	NS		NS		NS
Positive	0 (0)	0 (0)	0 (0)	5 (6.3)	2 (3.8)	7 (5.3)					
p53 mutation											
Negative	47 (97.9)	13 (86.7)	60 (95.2)	57 (71.3)	43 (81.1)	100 (75.2)	0.0025	18.1 (1.5-223.4)	0.0239	13.3 (1.4-129.3)	0.0256
Positive	1 (2.1)	2 (13.3)	3 (4.8)	23 (28.8)	10 (18.9)	33 (24.8)					
MSI											
MSS	39 (81.3)	12 (80.0)	51 (81.0)	65 (81.3)	43 (81.1)	108 (81.2)	NS		NS		NS
MSI-L	5 (10.4)	1 (6.7)	6 (9.5)	11 (13.8)	7 (13.2)	18 (13.5)					
MSI-H	4 (8.3)	2 (13.3)	6 (9.5)	4 (5.0)	3 (5.7)	7 (5.3)					

NOTE: Multivariate analysis B was done using data from the same tumors as multivariate analysis A based on the phenotypic marker expression pattern independently instead of the expressions of HGM, MUC6, MUC2, and CD10.
Abbreviations: LG, low grade; HG, high grade; MC, mucosal carcinoma; SMC, carcinoma with submucosal invasion; OR, odds ratio; 95% CI, 95% confidence interval; NA, not available; NS, not significant.

in 91 (46.4%), 107 (54.6%), 154 (78.6%), and 74 (37.8%) of the 196 early gastric differentiated-type tumors, respectively. Based on the expressions of these four markers, 32 (16.3%) tumors were classified as gastric phenotype tumors, 106 (54.1%) as gastric and intestinal mixed phenotype tumors, and 58 (29.6%) as intestinal phenotype tumors. Mutations in *APC, K-ras*, and *p53* were detected in 47 (24.0%), 7 (3.6%), and 36 (18.4%) of the 196 tumors. MSI for at least one marker (MSI-L and MSI-H) was detected in 37 (18.9%) of the 196 tumors. Among these 37 tumors, MSI for two or more markers (MSI-H) was detected in 13 (35.1%) tumors.

Relationships between clinicopathologic findings and phenotypic marker expressions in 63 gastric adenomas and 133 early differentiated-type carcinomas. The relationships between the clinicopathologic findings and the phenotypic marker expression in 63 gastric adenomas and 133 early differentiated-type carcinomas are shown in Table 2A and B. Among the 63 gastric adenomas, HGM-positive tumors, MUC6-positive tumors, and gastric or gastric and intestinal mixed phenotype tumors were significantly associated with a higher incidence of high-grade atypia compared with HGM-negative tumors, MUC6-negative tumors, and intestinal phenotype tumors [66.7% versus 19.3% (P = 0.0249), 45.5% versus 12.2% (P = 0.0051), and 50.0% and 39.1% versus 13.2% (P = 0.0319), respectively]. No significant differences in clinicopathologic findings according to the expressions of MUC2 and CD10 were observed.

Among the 133 early differentiated-type carcinomas, HGM-positive tumors were significantly associated with higher incidences of mixed-type tumors (differentiated-type carcinoma with an undifferentiated-type carcinoma component) and

Table 4. Relationships between phenotype marker expressions and genetic alterations in 63 gastric adenomas and 133 early differentiated-type carcinomas

	HGM expression		MUC6 expression		MUC2 expression	
	Negative	Positive	Negative	Positive	Negative	Positive
APC mutation						
Adenoma	23/57 (40.4)	2/6 (33.3)	20/41 (48.8)	5/22 (22.7)	3/7 (42.9)	22/56 (39.3)
Carcinoma*	16/48 (33.3)	6/85 (7.1)	10/48 (20.8)	12/85 (14.1)	5/35 (14.3)	17/98 (17.3)
Total†	39/105 (37.1)	8/91 (8.8)	30/89 (33.7)	17/107 (15.9)	8/42 (19.0)	39/154 (25.3)
K-ras mutation						
Adenoma	0/57 (0)	0/6 (0)	0/41 (0)	0/22 (0)	0/7 (0)	0/56 (0)
Carcinoma	4/48 (8.3)	3/85 (3.5)	1/48 (2.1)	6/85 (7.1)	1/35 (2.9)	6/98 (6.1)
Total	4/105 (3.9)	3/91 (3.3)	1/89 (1.1)	6/107 (5.6)	1/42 (2.4)	6/154 (3.9)
p53 mutation						
Adenoma	3/57 (5.3)	0/6 (0)	3/41 (7.3)	0/22 (0)	0/7 (0)	3/56 (5.4)
Carcinoma‡	15/48 (31.3)	18/85 (21.2)	13/48 (27.1)	20/85 (23.5)	7/35 (20.0)	26/98 (26.5)
Total	18/105 (17.1)	18/91 (19.8)	16/89 (18.0)	20/107 (18.7)	7/42 (16.7)	29/154 (18.8)
MSI (MSI-L or MSI-H)						
Adenoma	11/57 (19.3)	1/6 (16.7)	6/41 (14.6)	6/22 (27.3)	1/7 (14.3)	11/56 (19.6)
Carcinoma§	11/48 (22.9)	14/85 (16.5)	4/48 (8.3)	21/85 (24.7)	10/35 (28.6)	15/98 (15.3)
Total‖	22/105 (21.0)	15/91 (16.5)	10/89 (11.2)	27/107 (25.2)	11/42 (26.2)	26/154 (16.9)

*$P < 0.0001$ (HGM expression) and 0.0004 (CD10 expression).
†$P < 0.0001$ (HGM and CD10 expressions), $P = 0.0036$ (MUC6 expression), $P < 0.0001$ (CD10 expression), and $P = 0.0001$ (PMEP).
‡$P = 0.0069$ (CD10 expression).
§$P = 0.0217$ (MUC6 expression) and 0.0384 (CD10 expression).
‖$P = 0.0127$ (MUC6 expression).

lymph node metastasis compared with HGM-negative tumors [11.8% versus 0% ($P = 0.0137$) and 8.2% versus 0% ($P = 0.0487$), respectively]. MUC2-positive tumors were significantly associated with a higher incidence of mucosal carcinomas compared with MUC2-negative tumors (66.3% versus 42.9%; $P = 0.0149$). No significant differences in clinicopathologic findings according to the expressions of MUC6 and CD10 or the phenotypic marker expression patterns were observed.

Differences in clinicopathologic findings, phenotypic marker expressions, and genetic alterations between 63 gastric adenomas and 133 early differentiated-type carcinomas. The differences in the clinicopathologic findings, phenotypic marker expressions, and genetic alterations between 63 gastric adenomas and 133 early differentiated-type carcinomas are shown in Table 3. A univariate analysis revealed that the gastric adenomas were significantly correlated with a higher incidence of elevated-type tumors, a smaller tumor size, and higher incidences of HGM-negative tumors, MUC6-negative tumors, MUC2-positive tumors, CD10-positive tumors, intestinal phenotype tumors, tumors with *APC* mutations, and tumors without *p53* mutations compared with early differentiated-type carcinomas ($P < 0.0001$, 0.0001, 0.0001, 0.0002, 0.0187, 0.0001, 0.0001, 0.0006, and 0.0025, respectively). A multivariate analysis revealed significant differences in macroscopic type, tumor size, HGM expression, and *p53* mutation between gastric adenomas and early differentiated-type carcinomas ($P = 0.0001$, $P < 0.0001$, $P < 0.0005$, and $P < 0.0239$, respectively; multivariate analysis A). When data from the same tumors were analyzed based on the independent phenotypic marker expression patterns, significant differences in macroscopic type, tumor size, phenotypic marker expression pattern, and *p53* mutation were observed between gastric adenomas and early carcinomas ($P < 0.0001$, 0.0001, 0.0044, and 0.0256, respectively; multivariate analysis B).

Relationships between phenotype marker expressions and genetic alterations in 63 gastric adenomas and 133 early differentiated-type carcinomas. The relationships between the phenotypic marker expressions and genetic alterations in 63 gastric adenomas and 133 early differentiated-type carcinomas are shown in Table 4. HGM-positive tumors were significantly associated with a lower frequency of *APC* mutation compared with HGM-negative tumors in 133 early differentiated-type carcinomas and in all 196 tumors [7.1% versus 33.3% ($P < 0.0001$) and 8.8% versus 37.1% ($P < 0.0001$), respectively]. MUC6-positive tumors were significantly associated with a lower frequency of *APC* mutation compared with MUC6-negative tumors in all 196 tumors (15.9% versus 33.7%; $P = 0.0036$) and with a higher incidence of tumors with MSI-L and MSI-H in 133 early differentiated-type carcinomas and in all 196 tumors [24.7% versus 8.3% ($P = 0.0217$) and 25.2% versus 11.2% ($P = 0.0127$), respectively]. No significant differences in the incidence of each genetic alteration were observed between MUC2-positive tumors and MUC2-negative tumors. CD10-positive tumors were significantly associated with a higher frequency of *APC* mutation compared with CD10-negative tumors in 133 early differentiated-type carcinomas and in all 196 tumors [36.4% versus 10.0% ($P = 0.0004$) and 41.9% versus 13.1% ($P < 0.0001$), respectively], with a higher frequency of *p53* mutation in 133 early differentiated-type carcinomas (42.4% versus 19.0%; $P = 0.0135$), and with a lower incidence of tumors with MSI-L and MSI-H in 133 early differentiated-type carcinomas (6.1% versus 23.0%; $P = 0.0384$). With respect to the relationship between the phenotypic marker expression pattern and genetic alterations, gastric phenotype tumors and gastric and intestinal mixed phenotype tumors were significantly associated with a lower incidence of *APC* mutation compared with intestinal phenotype tumors in all 196 tumors (9.4% and 17.9% versus 41.4%; $P = 0.0016$ and 0.0011, respectively).

Table 4. Relationships between phenotype marker expressions and genetic alterations in 63 gastric adenomas and 133 early differentiated-type carcinomas (Cont'd)

CD10 expression		PMEP		
Negative	Positive	G	GI	I
6/22 (27.1)	19/41 (46.3)	0/2 (0)	6/23 (26.1)	19/38 (50.0)
10/100 (10.0)	12/33 (36.4)	3/30 (10.0)	13/83 (15.7)	5/20 (25.0)
16/122 (13.1)	31/74 (41.9)	3/32 (9.4)	19/106 (17.9)	24/58 (41.4)
0/22 (0)	0/41 (0)	0/2 (0)	0/23 (0)	0/38 (0)
5/100 (5.0)	2/33 (6.1)	1/30 (3.3)	5/83 (6.0)	1/20 (5.0)
5/122 (4.1)	2/74 (2.7)	1/32 (3.1)	5/106 (4.7)	1/58 (1.7)
1/22 (4.5)	2/41 (4.9)	0/2 (0)	0/23 (0)	3/38 (7.9)
19/100 (19.0)	14/33 (42.4)	6/30 (20.0)	19/83 (22.9)	8/20 (40.0)
20/122 (16.4)	16/74 (21.6)	6/32 (18.8)	19/106 (17.9)	11/58 (19.0)
5/22 (22.7)	7/41 (17.1)	1/2 (50.0)	5/23 (21.7)	6/38 (15.8)
23/100 (23.0)	2/33 (6.1)	9/30 (30.0)	13/83 (15.7)	3/20 (15.0)
28/122 (12.2)	9/74 (12.2)	10/32 (31.3)	18/106 (17.0)	9/58 (15.5)

Clinicopathologic findings, phenotypic marker expressions, and genetic alterations in 24 follow-up cases of gastric adenoma. We next examined the clinicopathologic findings, phenotypic marker expressions, and genetic alterations in 24 follow-up cases of gastric adenoma. All 24 tumors were diagnosed as gastric adenoma by histologic examination of the initial biopsy specimen obtained by endoscopic examination. In 5 of these 24 cases, a histologic examination of the final biopsy specimens, endoscopically resected lesions, or surgical specimens revealed a malignant transformation of gastric adenoma at the end of the follow-up period; in 19 cases, no evidence of adenocarcinoma was found based on a histologic examination of the final biopsy specimens at the end of the follow-up period. The clinicopathologic findings, phenotypic marker expressions, and genetic alterations of the 24 gastric adenomas at the initial diagnosis are shown in Table 5A, whereas those of the 16 tumors in which phenotypic marker expressions and genetic alterations were determined at the final diagnosis are shown in Table 5B. The phenotypic marker expressions and genetic alterations in the eight other cases could not be analyzed because adequate tissue samples were not available.

Based on the analysis of the 24 gastric adenoma specimens obtained at the initial diagnosis, the gastric adenomas that progressed to adenocarcinomas were significantly associated with a higher incidence of gastric adenoma with severe atypia in histologic grade ($P = 0.0065$), a larger tumor size ($P = 0.0073$), and lower incidences of intestinal phenotype tumor ($P = 0.0474$) and tumors with *APC* mutation ($P = 0.0411$) compared with gastric adenomas that did not progress to carcinomas. Among the five gastric adenomas that progressed to differentiated-type carcinoma, four tumors were gastric and intestinal mixed phenotype tumors and none of the tumors carried an *APC* mutation. No malignant transformation was found in intestinal phenotype adenomas with *APC* mutation among the 24 follow-up cases of gastric adenoma.

With respect to the transformation in phenotypic marker expression, mutations in *APC*, *K-ras*, and *p53*, and the MSI status between specimens at the initial diagnosis and those at the final diagnosis among the seven tumors without CD10 expression at the initial diagnosis, CD10 expression was shown in four tumor specimens at the final diagnosis (cases 1, 11, 18, and 23). Among the nine tumors without *APC* mutation at the initial diagnosis, *APC* mutation was detected in two tumor specimens at the final diagnosis (cases 1 and 18). These two tumors were accompanied by a phenotypic shift (from CD10 negative to CD10 positive) during the follow-up period. Among the 14 tumors without MSI at the initial diagnosis, MSI was detected in three tumor specimens at the final diagnosis (cases 11, 14, and 18). No differences in the expressions of HGM, MUC6, and MUC2, the phenotypic marker expression pattern, *K-ras* mutation, and *p53* mutation were observed between specimens obtained at the initial diagnosis and those obtained at the final diagnosis.

Discussion

Our present results revealed that the expressions of HGM and MUC6 and the gastric phenotype were associated with high-grade atypia in 63 gastric adenomas. In 133 early differentiated-type carcinomas, HGM expression was associated with mixed-type tumors and lymph node metastasis. MUC2 expression was inversely associated with invasion to the submucosa. These findings show distinct differences in tumor aggressiveness according to the phenotypic marker expressions of early-stage gastric differentiated-type tumors. Differentiated-type gastric carcinomas with a gastric phenotype have been suggested to be more likely to transform into undifferentiated-type carcinoma and to show infiltrative growth to deeper layers of the invasion of the surrounding structures through the loss of *E-cadherin* function compared with those with an intestinal phenotype (7, 10, 12, 13, 20). Mixed-type tumors have been considered to represent a progressive loss of glandular structure and a histologic transformation from differentiated-type to undifferentiated-type carcinoma during the progression of the tumor. Therefore, our present results support previous findings showing that phenotypic marker expression is associated with histologic transformation during the early stage of gastric tumorigenesis. Koseki et al. (12) reported that the gastric

phenotype is an independent factor associated with lymph node metastasis among early gastric carcinomas. Among advanced gastric carcinomas, gastric phenotype tumors have been also associated with a greater malignant potential during invasion and metastasis compared with tumors of other phenotypes (10, 12). Therefore, phenotypic marker expression could reflect tumor aggressiveness in gastric adenomas and carcinomas.

In previous reports on differences in phenotypic marker expressions and genetic alterations among gastric adenomas and differentiated-type carcinomas, the majority of gastric adenomas were reported to be intestinal phenotype, whereas gastric carcinomas were reported to mainly express gastric phenotypic markers (14). *APC* mutation was reported in up to 40% of gastric adenomas but only rarely in carcinoma. *p53* mutation was reported in 30% of gastric carcinomas but only rarely in gastric adenomas (11, 32, 36). However, as far as we are aware, no studies involving a large number of cases have been published in which gastric adenomas and early differentiated-type carcinomas were compared in multivariate analyses examining clinicopathologic features, phenotypic marker expression, and genetic alterations (Table 1). In the present study, a multivariate analysis revealed significant differences in macroscopic type, tumor size, HGM expression (or the phenotypic marker expression pattern), and *p53* mutation between gastric adenomas and early carcinomas. These findings show that macroscopic type, tumor size, the phenotypic marker expression, and *p53* mutation are the most important factors for differentiating between gastric adenoma and differentiated-type carcinoma.

Table 5. Clinicopathologic findings, phenotypic marker expressions, and genetic alterations in 24 follow-up cases with gastric adenoma

A. Clinicopathologic findings, phenotypic marker expressions, and genetic alterations at the initial diagnosis in 24 follow-up cases with gastric adenoma

Cases	Initial diagnosis	Final diagnosis	Follow-up time (mo)	Macroscopic type	Tumor size (mm)	HGM expression
1	High	Carcinoma	6	Flat	10	–
2	High	Carcinoma	6	Elevated	18	–
3	High	Carcinoma	7	Elevated	15	–
4	High	Carcinoma	8	depressed	15	+
5	High	Carcinoma	10	Elevated	15	–
6	Low	High	6	Elevated	15	–
7	Low	Low	6	Elevated	8	–
8	Low	Low	6	Elevated	10	–
9	Low	Low	7	Elevated	3	+
10	High	High	7	Elevated	18	–
11	High	High	8	Depressed	8	–
12	Low	Low	8	Elevated	4	–
13	Low	Low	9	Elevated	14	–
14	Low	Low	11	Elevated	5	–
15	Low	Low	11	Elevated	5	–
16	Low	Low	12	Elevated	7	–
17	Low	High	14	Elevated	10	–
18	Low	Low	20	Elevated	6	–
19	Low	Low	24	Elevated	7	–
20	Low	Low	24	Elevated	3	–
21	Low	Low	25	Elevated	3	–
22	Low	Low	32	Elevated	3	–
23	Low	Low	54	Elevated	11	–
24	Low	Low	55	Elevated	4	–

B. Clinicopathologic findings, phenotypic marker expressions, and genetic alterations at the final diagnosis in 24 follow-up cases with gastric adenoma

Cases	Macroscopic type	Tumor size (mm)	HGM expression	MUC6 expression	MUC2 expression	CD10 expression
1	Flat	10	–	+	+	+
2	Elevated	18	–	+	+	+
3	Elevated	15	–	–	+	+
4	Depressed	15	+	–	+	–
5	Elevated	15	–	+	+	–
6	Elevated	15	–	–	+	+
8	Elevated	10	–	–	+	–
9	Elevated	3	+	+	+	+
11	Depressed	12	–	+	+	+
14	Elevated	5	–	–	+	+
16	Elevated	7	–	–	+	+
18	Elevated	6	–	+	–	+
21	Elevated	5	–	–	+	+
22	Elevated	3	–	–	+	+
23	Elevated	11	–	+	+	+
24	Elevated	4	–	–	+	+

There have been several reports on the genetic alterations in gastric adenomas and differentiated-type carcinomas. However, genetic alterations according to phenotypic marker expression patterns have been examined in only a few studies on gastric adenoma and early differentiated-type carcinoma, and the results of these studies remain to be clarified (9, 11, 17). Our present results revealed significant associations between phenotypic marker expression patterns and genetic alterations in early gastric differentiated-type tumors. *APC* mutation was significantly associated with CD10 expression and the intestinal phenotype and inversely associated with the expressions of HGM and MUC6. *p53* mutation was significantly associated with CD10 expression. MSI status was significantly associated with MUC6 expression. These findings suggest that phenotypic marker expression is closely related to genetic alterations in the incipient phase of gastric tumorigenesis. Differentiation to gastric pyloric gland cells is thought to be related to MSI, whereas differentiation to intestinal epithelial cells, especially absorptive cells, is thought to be related to mutations in *APC* or *p53*.

The adenoma-carcinoma sequence has been analyzed in colorectal cancer, and this theory is generally accepted (37). Nevertheless, only 11% to 40% of gastric adenomas progress to differentiated-type carcinomas. Previous studies have reported that the risk of malignant transformation in gastric adenomas was related to clinicopathologic characteristics, such as a large tumor size and high-grade atypia (38). However, to the best of our knowledge, no previous study has examined phenotypic marker expression and genetic alterations in follow-up cases with gastric adenoma. In the present study, the gastric adenomas that progressed to carcinomas were significantly associated with

Table 5. Clinicopathologic findings, phenotypic marker expressions, and genetic alterations in 24 follow-up cases with gastric adenoma (Cont'd)

A. Clinicopathologic findings, phenotypic marker expressions, and genetic alterations at the initial diagnosis in 24 follow-up cases with gastric adenoma

MUC6 expression	MUC2 expression	CD10 expression	PMEP	*APC* mutation	*K-ras* mutation	*p53* mutation	MSI status
+	+	–	GI	–	–	–	MSS
+	+	+	GI	–	–	–	MSS
–	+	+	I	–	–	–	MSS
–	+	–	GI	–	–	–	MSI-L
+	+	–	GI	–	–	–	MSI-H
–	+	+	I	+	–	–	MSS
–	–	+	I	+	–	–	MSS
–	+	–	I	+	–	–	MSS
+	+	+	GI	–	–	–	MSS
–	+	–	I	–	–	+	MSI-L
+	+	–	GI	+	–	–	MSS
–	+	+	I	+	–	–	MSS
–	+	–	GI	–	–	–	MSS
–	+	+	I	+	–	–	MSS
–	+	+	I	–	–	–	MSS
–	+	+	I	+	–	–	MSS
–	+	+	I	–	–	–	MSS
+	–	–	G	–	–	–	MSS
–	–	–	I	+	–	–	MSS
–	+	+	I	+	–	–	MSS
–	+	+	I	–	–	–	MSS
–	+	+	I	+	–	–	MSS
+	+	–	GI	–	–	–	MSS
–	+	+	I	+	–	–	MSS

B. Clinicopathologic findings, phenotypic marker expressions, and genetic alterations at the final diagnosis in 24 follow-up cases with gastric adenoma

PMEP	*APC* mutation	*K-ras* mutation	*p53* mutation	MSI status
GI	+	–	–	MSS
GI	–	–	–	MSS
I	–	–	–	MSS
GI	–	–	–	MSI-L
GI	–	–	–	MSI-H
I	+	–	–	MSS
I	+	–	–	MSS
GI	–	–	–	MSS
GI	+	–	–	MSI-L
I	+	–		MSI-L
I	+	–	–	MSS
GI	+	–	–	MSI-L
I	–	–	–	MSS
I	+	–	–	MSS
GI	–	–	–	MSS
I	+	–	–	MSS

a larger tumor size and severe atypia and were inversely associated with an intestinal phenotype and *APC* mutation compared with tumors that did not progress to carcinomas. Among our follow-up cases, malignant transformation did not occur in any of the intestinal phenotype adenomas with *APC* mutation, which are characteristics of gastric adenoma. These findings suggest that not only clinicopathologic findings, such as tumor size and degree of atypia, but also phenotypic marker expression and *APC* mutation are important risk factors predicting malignant transformation in gastric adenomas. For patients with a high risk of malignant transformation, such as gastric adenomas with gastric phenotype and without *APC* mutation, endoscopic mucosal resection may be recommended.

Among our follow-up cases, CD10 expression was shown at the final diagnosis in four of seven gastric adenomas without CD10 expression at the initial diagnosis, reflecting a phenotypic shift from a gastric phenotype to an intestinal phenotype with time. The phenotypic shift from a gastric to an intestinal phenotype with progression in gastric tumors, which is seen in gastric nonneoplastic mucosa with intestinal metaplasia, has also been previously shown (14, 18, 19, 39). Tsukashita et al. (14) speculated that the phenotypic shift from a gastric to an intestinal phenotype might represent the natural course of both gastric nonneoplastic mucosa and differentiated-type tumors. Furthermore, in our follow-up cases, two tumors in which *APC* mutations were not detected at the initial diagnosis but were detected at the final diagnosis were accompanied by a phenotypic shift from a gastric to an intestinal phenotype during the follow-up period. Therefore, the differentiation to intestinal absorptive cells, which might be associated with *APC* mutation, was thought to have occurred over time in these cases. These findings support an association between *APC* mutation and CD10 expression or the intestinal phenotype in early gastric differentiated-type tumors.

Our present findings show distinct differences in the biological behaviors of tumors with different phenotypic marker expression patterns in early gastric differentiated-type tumors. We previously reported that patients with gastric phenotype tumors have a poorer prognosis than those with intestinal phenotype tumors among patients with advanced gastric carcinoma (13). We also reported that the majority of peritoneal recurrences after a curative resection for gastric carcinoma occurred in gastric phenotype tumors, especially HGM-positive tumors, whereas hematogenous recurrence occurred more frequently in MUC2-negative and CD10-positive tumors (16). These differences in the biological behaviors of tumors with different phenotypic marker expression patterns suggest the actions of different genetic alterations. We recently reported that chromosomal changes detected using a comparative genomic hybridization technique differ considerably according to the phenotypic marker expression patterns of gastric differentiated-type carcinomas (20). As described above, our present findings show different patterns of genetic alterations according to the phenotypic marker expression pattern of the tumor in early gastric differentiated-type tumors. Previous molecular genetic studies have shown that gastric tumorigenesis is a multistep process involving the accumulation of genetic alterations (3, 21). Therefore, the present study suggests that different genetic pathways, which are related to phenotypic marker expression patterns, may exist during the incipient phase of gastric tumorigenesis, leading to different biological behaviors.

In conclusion, our present findings show that phenotypic marker expression patterns reflect the biological behaviors of early gastric differentiated-type tumors. Differences in the biological behaviors of tumors with different phenotypes may result from differences in the genetic background during the incipient phase of gastric tumorigenesis.

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