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Genetic Expression Pattern of Gastric Carcinomas According to Cellular Mucin Phenotypes

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Background : Gastric carcinomas (GCs) have recently been reclassified according to the mucin phenotypes. We aimed to characterize the relationship between the mucin phenotypes and the genetic alterations or the clinicopathologic parameters of GCs. **Methods :** Immunohistochemistry was performed for MUC1, MUC5AC, MUC6, MUC2, CD10, p53, hMLH1, C-erbB2 and E-cadherin in 150 GCs. The mucin phenotypes of the GCs were classified as 4 phenotypes: gastric, intestinal, mixed and unclassified. **Results :** MUC1, MUC5AC, MUC6, MUC2 and CD10 were expressed in 63.3%, 42.7%, 14.0%, 24.7% and 14.0% of the GCs, respectively. The mucin phenotypes of the GCs corresponded to the gastric type in 31.3%, the intestinal type in 20.0%, the mixed type in 15.3% and the unclassified type in 33.3%. The incidence of a p53 overexpression was higher in the gastric or mixed phenotype than in the intestinal or unclassified phenotype. MUC5AC expression, p53 overexpression and the gastric or mixed phenotype were associated with poor patient survival by multivariate analysis. **Conclusion :** This study suggests the gastric or mixed mucin phenotype may more likely go through the p53 pathway in carcinogenesis and the mucin phenotype may be considered as a prognostic indicator.

Key Words : Stomach; Carcinoma; Mucins; TP53 protein; hMLH1 protein; ERBB2 protein; E-cadherin

Gastric carcinomas (GCs) reveal diverse histopathologic or genetic alterations within the same tumor as well as among different tumors.[1,2] GCs have traditionally been classified into two main types, the so-called intestinal and diffuse types, based on the tendency toward gland-formation. These types closely correspond to the differentiated- and undifferentiated- types, respectively.[3] The differentiated- and undifferentiated-type carcinomas not only show different epidemiological characteristics but they may also be derive from different genetic pathways.[4]

The recent use of immunohistochemical staining techniques specific for gastric- and intestinal type mucins has led to histological reclassification of the phenotype of each tumor.[2,5-13] At least 9 mucin genes (MUC1, 2, 3, 4, 5AC, 5B, 6, 7 and 8) have been identified by molecular biology techniques.[13,14] The expression of mucin genes is relatively specific to the tissue type. MUC1, which is a membrane-bound mucin, is expressed in many normal epithelia, including those of the bronchus, colon, ileum, lung and stomach. In the stomach, MUC1 is expressed in the foveolar epithelium of the gastric antrum and in the oxyntic glands of the gastric body.[15] MUC5AC and MUC6 are expressed in the superficial foveolar epithelium and the pyloric glands of the gastric mucosa, respectively.[15,16] MUC2 is expressed predominantly in goblet cells of the intestinal mucosa or in the intestinal metaplastic cells of the stomach, and it is usually not expressed in normal gastric mucosa.[15] CD10 is known as a common acute lymphocytic leukemia antigen. However, CD10 is also expressed in the brush border of the complete-type intestinal metaplasia.[17] The recognition of the brush border by CD10 immunostaining proved useful for making an accurate diagnosis of the mucin phenotype of GC.[1,5] Alterations of the mucin expression are known to take place in gastric carcinogenesis.[18-20]

Gastric cancers involve diverse alterations of various oncogenes, tumor suppressor genes and DNA mismatch repair (MMR) genes. Common or different gene changes may be observed in the different histogenetic phenotypes of cancer.[4,21]

Inactivation of the p53 gene is a common event in the car-

cinogenesis of various organs, including the stomach, and this is considered as a marker of a poor prognosis.[21] The accumulation of p53 protein by immunohistochemistry is useful for screening the p53 gene. Germline defects in the MMR gene have been frequently found in hereditary non-polyposis colorectal cancer (HNPCC). Up to now, five DNA repair genes (hMLH1, hMSH2, hMSH6, hPMS1, hPMS2) have been found to be involved in HNPCC. More than 90% of the detectable mutations in the HNPCC kindred are in hMLH1 and hMSH2.[22,23] Recently, mutations in the MMR genes were also found in sporadic cancers of various organs, including the stomach.[22-24]

The calcium-dependent homophilic cell adhesion molecule E-cadherin and the associated catenins link polarized epithelial cells to maintain the structural integrity of an epithelial monolayer.[25] Structural abnormalities of E-cadherin that are caused by gene mutations have been shown to disrupt E-cadherin-mediated intercellular adhesion, causing loose cell-to-cell adhesion in tumor cells.[26] In general, E-cadherin immunoreactivity is often reduced or lost in the less differentiated and invasive carcinomas.[1] The c-erb-B2, human epidermal growth factor receptor 2, is a transmembrane glycoprotein that's involved in the control of cell growth. The c-erb-B2 gene is amplified in approximately 25% of breast cancers and also in human adenocarcinomas that arise within the ovary, lung, stomach and salivary glands. The amplification of c-erb B2 gene can be determined either by evaluating the protein content with performing immunohistochemistry or by determining the gene copy number by using fluorescence in situ hybridization.[27]

This study aimed at characterizing the mucin expression profile of MUC1, MUC5AC, MUC6, MUC2 and CD10 in GCs, at classifying GCs according to the mucin phenotype and at analyzing the relationship between the mucin phenotypes and the genetic alteration of p53, hMLH1, E-cadherin and c-erb-B2, or the clinicopathologic findings.

MATERIALS AND METHODS

Samples

A total of 150 surgically resected GCs were selected from the files of the Department of Pathology, Kyungpook National University Hospital and Dankook University Hospital, between January 1998 and April 2001. The clinical information and pathologic reports were reviewed for each case. The GCs were classified according to Lauren's classification as the diffuse, intestinal and mixed types. The mean age of the patients was 58.08 years (range: 30-83 years). The clinicopathologic features of the 150 patients are presented in Table 1. The clinical outcome of the patients was followed from the date of surgery to the date of death or to May 20 2003. The follow-up period ranged from 1 month to approximately 64 months (mean: 28 months).

Immunohistochemistry

Core tissue biopsies (2 mm in diameter) were taken from individual paraffin-embedded gastric cancers and then arranged in a new recipient paraffin block (tissue-array block). Each tissue-array block contained about 40 cases, with a total of 4

Table 1. Clinicopathologic features of the 150 patients

Characteristics	Number of cases (%)
Gender (male:female)	115 (76.6):35 (23.3)
Histologic type (diffuse:intestinal:mixed)	68 (45.3):78 (52.0):4 (2.6)
Depth of invasion (T1:T2:T3:T4)	42 (28.0):22 (14.6):59 (39.3):27 (4.6)
Lymph node metastasis (negative:positive)	62 (41.3):88 (58.6)
Stage (I:II:II:IV)	54 (36.0):41 (27.3):40 (26.6):15 (10.0)

SD, standard deviation; T1, tumor has invaded lamina propria or submucosa; T2, tumor has invaded muscularis propria or subserosa; T3, tumor has invaded serosa; T4, tumor has invaded adjacent structures.

Table 2. Primary antibodies used for immunohistochemistry

Primary antibody	Clone	Manufacture	Dilution	Antigen retrieval
MUC1	HMFG	Novocastra, Newcastle, UK	1:100	Microwave
MUC5AC	45M1	Novocastra, Newcastle, UK	1:400	Microwave
MUC6	CLH5	Lab Vision, Fremont, CA, USA	1: 50	Microwave
MUC2	Ccp58	Novocastra, Newcastle, UK	1:200	Microwave
CD10	56C6	Novocastra, Newcastle, UK	1:50	Microwave
p53	DO-7	Novocastra, Newcastle, UK	1:100	Microwave
MLH1	G168-15	PharMingen, San Diego, CA, USA	1:10	Microwave
C-erbB2	polyclonal	DAKO, Denmark	1:200	Microwave
E-cadherin	clone36	Transduction Lab., Lexington, KY, USA	1:50	Microwave

blocks of tissue array. The tissue microarray slides were deparaffinized with xylene, rehydrated with ethanol, washed and then subjected to microwave retrieval in citrate buffer. The sections were then immersed in 3% hydrogen peroxide to block endogenous peroxidase activity and they were next incubated with the primary antibodies. The primary antibodies used are listed in Table 2. The expressions were detected using a CAP-Plus kit (Zymed, South San Francisco, CA, USA). Antibody-antigen reactivity was visualized using diaminobenzidine and counterstaining with Mayer's hematoxylin.

Assessment of immunohistochemical staining

The results of immunostaining for MUC1, MUC5AC, MUC6, MUC2 and CD10 were considered to be positive if more than 10% of the tumor cells were stained. The normal staining pattern for MUC1 is membranous and the staining pattern for MUC5AC and MUC2 is cytoplasmic. CD10 stains along the brush borders of the luminal surface. The results were considered to be negative when less than 10% of the tumor cells were stained. The MUC6 and MUC5AC expressions were examined as markers of the gastric phenotypes and the MUC2 and CD10 expressions were examined as markers of the intestinal phenotypes. The cellular mucin phenotypes of the GCs were classified according to the combined expression patterns of the gastric markers and intestinal markers as 4 phenotypes: the gastric type (tumor cells were positive for either MUC6 or MUC5AC, and negative for both MUC2 and CD10), the intestinal type (tumor cells were positive for either MUC2 or CD10, and negative for both MUC6 and MUC5AC), the mixed type (tumor cells were positive for both gastric and intestinal markers), and the unclassified phenotype (tumor cells were negative for both gastric and intestinal markers).

The normal staining pattern of p53 and hMLH1 is nuclear staining. Overexpression of p53 protein was defined as when more than 10% of the tumor cells revealed positive staining. For the evaluation of the hMLH1 expression, non-neoplastic epithelial and stromal cells were used as positive controls. Those cases with definite nuclear staining in more than 70% of the tumor cells were categorized as positive, and those cases with definite nuclear staining in less than 30% of the tumor cells were categorized as negative. C-erb B2 overexpression was defined as membranous staining in more than 10% of the tumor cells. The evaluation of the E-cadherin expression was categorized as normal or aberrant. Only a membranous pattern, which stained as strongly as the normal epithelial cells, was judged as normal. The aberrant expression of E-cadherin was defined as either the absence of membranous staining or less than 30% membranous staining of the examined tumor cells.

Statistical analysis

We performed statistical analyses using the chi-square test. Survival curves were plotted using the Kaplan-Meier method, and differences between the survival curves were tested using the log-rank test. Multivariate analysis for the covariates that showed statistical significance on the univariate analysis was performed using the Cox proportional hazards model. The results were considered to be statistically significant when p values were less than 0.05. All statistical analyses were conducted using the SPSS 11.0 statistical software program (SPSS, Chicago, IL, USA).

RESULTS

Expression of MUC1, MUC5AC, MUC6, MUC2 and CD10

The microscopic features of the immunohistochemistry for MUC1, MUC5AC, MUC6, MUC2 and CD10 are presented in Fig. 1. In the non-neoplastic gastric mucosa, MUC1 and MUC5AC mucins were expressed in the foveolar epithelial cells, MUC6 in the pyloric glands and MUC2 in the goblet cells of the intestinal metaplasia. Of the 150 cases of GCs, MUC1, MUC5AC, MUC6, MUC2 and CD10 were expressed in 95 (63.3%), 64 (42.7%), 21 (14.0%), 37 (24.7%) and 21 (14.0%) cases, respectively. The relationship between the mucin phenotypes and the clinicopathologic features are presented in Table 3. MUC1 was more frequently observed in the intestinal type than in the other histologic types according to Lauren's classification (p=0.02). The CD10 expression was more frequent in the intestinal type than the other histologic types with borderline significance (p=0.052). A MUC5AC expression was more frequently observed in early gastric cancer than in the advanced gastric cancer (p=0.0001). The MUC5AC expression was inversely correlated with lymph node metastasis with borderline significance (p=0.06).

Assessment of cellular mucin phenotypes

Taking into account the combinations of the expression of the gastric (MUC6 and MUC5AC) and intestinal (MUC2 and CD10) markers, the 150 GCs were classified as four cellular

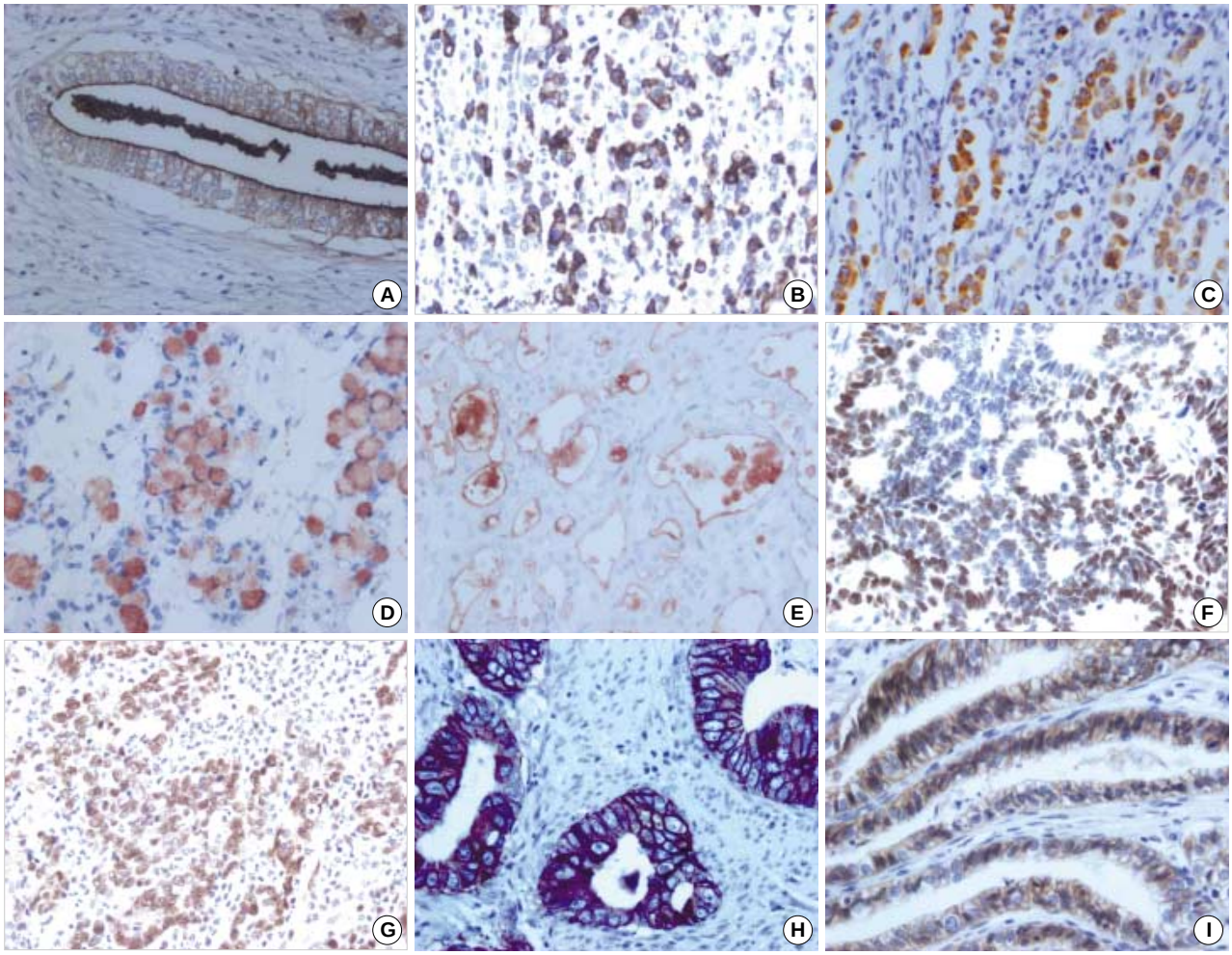

Fig. 1. Microscopic features of immunohistochemistry. The immunostaining for MUC1 reveals membranous staining in tumor cells (A). The staining for MUC5AC (B), MUC6 (C) and MUC2 (D) reveals cytoplasmic staining in tumor cells. CD10 is expressed along the brush border of luminal surfaces in tumor cells (E). The staining for p53 (F) and hMLH1 (G) reveals nuclear staining in tumor cells. c-erb B2 immunostaining shows a complete, intense membranous pattern in tumor cells (H). The staining for E-cadherin reveals strong membranous staining in tumor cells (I).

mucin phenotypes as follows: the gastric phenotype in 47 (31.3 %) cases, the intestinal phenotype in 30 (20%) cases, the mixed phenotype in 23 (15.3%) cases and the unclassified phenotype in 50 (33.3%) cases. The relationship between the mucin phenotypes and the clinicopathologic features are presented in Table 4. The cellular mucin phenotype was not consistent with the histologic type according to Lauren's classification. The mucin phenotypes did not correlate with any of the clinicopathologic features.

Expression of the p53, hMLH1, E-cadherin and c-erb-B2

The microscopic features of immunohistochemistry for p53, hMLH1, c-erbB2 and E-cadherin are presented in Fig. 1. The p53 overexpression, the loss of the hMLH1 expression, c-erbB2 overexpression and an aberrant E-cadherin expression were observed in 76 cases (50.7%), 30 cases (20%), 13 cases (8.9%) and 55 cases (36.7%), respectively. The incidence of an aberrant E-cadherin expression was significantly higher in the diffuse type than in the other histologic types according to Lauren's classification ($p=0.002$).

Relation between the mucin phenotype and genetic alterations

The relation between the mucin expression and alterations of p53, hMLH1, E-cadherin and c-erb-B2 is presented in Table 5. The MUC1, MUC5AC and CD10 expressions were correlated

Table 3. Comparison between mucin expression and clinicopathologic features

	No. of cases	MUC1		MUC5AC		MUC6		MUC2		CD10	
		+ (%)	p value	+ (%)	p value	+ (%)	p value	+ (%)	p value	+ (%)	p value
Lauren's classification			0.02		NS		NS		NS		0.052
Diffuse	68	36 (52.9)		29 (42.6)		7 (10.2)		17 (25.0)		5 (62.5)	
Intestinal	78	55 (70.5)		34 (43.5)		14 (17.9)		19 (24.3)		16 (20.5)	
Mixed	4	2 (50.0)		1 (25.0)		0 (0)		1 (25.0)		0 (0)	
Depth of invasion			NS		0.0001		NS		NS		NS
T1	42	26 (61.9)		28 (66.6)		10 (23.8)		13 (30.9)		7 (16.6)	
T2-T4	108	69 (63.8)		36 (33.3)		11 (10.1)		24 (22.2)		14 (12.9)	
Lymph node metastasis			NS		0.06		NS		NS		NS
Absent	62	41 (66.1)		32 (51.6)		9 (14.5)		15 (24.1)		8 (12.9)	
Present	88	54 (61.3)		32 (36.3)		12 (13.6)		22 (25.0)		13 (14.7)	
Stage			NS		NS		NS		NS		NS
I	54	37 (68.5)		27 (50.0)		10 (18.5)		11 (20.3)		7 (12.9)	
II	41	24 (58.5)		16 (39.0)		7 (17.0)		14 (34.1)		6 (14.6)	
III	40	23 (57.5)		15 (37.5)		2 (5.0)		9 (22.5)		7 (17.5)	
IV	15	11 (73.3)		6 (40.0)		2 (13.3)		3 (20.0)		1 (6.6)	
Total	150	95 (63.3)		64 (42.6)		21 (14.0)		37 (24.6)		21 (14.0)	

+: Number of positive cases; T1, tumor has invaded lamina propria or submucosa; T2, tumor has invaded muscularis propria or subserosa; T3, tumor has invaded serosa; T4, tumor has invaded adjacent structures.

Table 4. Comparison between cellular mucin phenotypes and clinicopathologic features

Clinicopathologic features	No. of cases	Mucin phenotype of the tumor				p value
		Gastric (%)	Intestinal (%)	Mixed (%)	Unclassified (%)	
Lauren's classification						NS
Diffuse	68	24 (35.2)	13 (19.1)	8 (11.7)	23 (33.8)	
Intestinal	78	22 (28.2)	16 (20.5)	15 (19.2)	25 (32.0)	
Mixed	4	1 (25.0)	1 (25.0)	0 (0)	2 (50.0)	
Depth of invasion						NS
T1	42	22 (52.3)	10 (23.8)	7 (16.6)	15 (35.7)	
T2-T4	108	25 (23.1)	20 (18.5)	15 (13.8)	35 (32.4)	
Lymph node metastasis						NS
Absence	62	23 (37.0)	11 (18.3)	9 (14.5)	19 (30.6)	
Presence	88	24 (27.2)	19 (21.5)	14 (15.9)	31 (35.2)	
Stage						NS
I	54	22 (40.7)	10 (18.5)	7 (12.9)	15 (27.7)	
II	41	9 (21.9)	8 (19.5)	9 (21.9)	15 (36.5)	
III	40	10 (25.0)	10 (25.0)	5 (12.5)	15 (37.5)	
IV	15	6 (40.0)	12 (8.0)	2 (13.3)	5 (33.3)	
Total	150	47 (31.3)	30 (20.0)	23 (15.3)	50 (33.3)	

NS, not significant; T1, tumor has invaded lamina propria or submucosa; T2, tumor has invaded muscularis propria or subserosa; T3, tumor has invaded serosa; T4, tumor has invaded adjacent structures.

with a p53 overexpression (p=0.003, 0.03 and 0.04, respectively). A MUC1 expression was inversely correlated with an aberrant E-cadherin expression (p=0.003). A MUC6 expression was inversely correlated with the loss of the hMLH1 expression with borderline significance (p=0.06). An aberrant E-cadherin expression was inversely correlated with a CD10 expression with borderline significance (p=0.06).

The relation between the cellular mucin phenotypes and the p53, hMLH1, E-cadherin and c-erb-B2 expressions is presented in Table 6. The incidence of p53 overexpression was higher in the mixed gastric and intestinal phenotype than in the other mucin phenotypes (p=0.05). When the mucin phenotypes were divided into two groups, i.e., the gastric or mixed phenotype, and the intestinal or unclassified phenotype, the group of the gastric or mixed phenotype was associated with a p53 overexpression with statistical significance (p=0.032) and with an aberrant E-cadherin expression with borderline significance (p=0.089). There was no relation between the mucin pheno-

Table 5. The relation between mucin expression and genetic alteration

	No. of cases	MUC1		MUC5AC		MUC6		MUC2		CD10	
		+ (%)	p value	+ (%)	p value	+ (%)	p value	+ (%)	p value	+ (%)	p value
P53 overexpression			0.003		0.03		NS		NS		0.04
Absent	74	38 (51.3)		25 (33.7)		10 (13.5)		18 (24.3)		6 (8.1)	
Present	76	57 (75.0)		39 (51.3)		11 (14.4)		19 (25.0)		15 (19.7)	
Loss of hMLH1 expression			NS		NS		0.06		NS		NS
Absent	120	73 (60.8)		51 (42.5)		20 (16.6)		32 (26.6)		15 (12.5)	
Present	30	22 (73.3)		13 (43.3)		1 (3.3)		5 (16.6)		6 (20.0)	
c-ErbB2 overexpression*			NS		NS		NS		NS		NS
Absent	133	35 (26.3)		25 (18.7)		11 (8.2)		15 (11.2)		19 (14.2)	
Present	13	2 (15.3)		1 (7.6)		10 (76.9)		2 (15.3)		2 (15.3)	
E-cadherin expression†			0.003		NS		NS		NS		0.06
Normal	93	68 (73.1)		35 (36.8)		19 (20.4)		24 (25.8)		17 (18.2)	
Aberrant	55	27 (49.0)		28 (50.9)		2 (3.6)		12 (21.8)		4 (7.2)	
Total	150	95 (63.3)		64 (42.6)		21 (14.0)		37 (24.6)		21 (14.0)	

+, Number of positive cases; NS, not significant; *4 cases are missing; †2 cases are missing.

Table 6. Comparison between cellular mucin phenotypes and genetic alteration

Clinicopathologic features	No. of cases	Mucin phenotype of the tumor				p value
		Gastric (%)	Intestinal (%)	Mixed (%)	Unclassified (%)	
P53 overexpression						0.05
Absent	74	22 (29.7)	16 (21.6)	6 (8.1)	30 (40.5)	
Present	76	25 (32.8)	14 (18.4)	17 (22.3)	20 (26.3)	
Loss of hMLH1 expression						NS
Absent	120	37 (30.8)	24 (20.0)	19 (15.8)	40 (33.3)	
Present	30	10 (33.3)	6 (20.0)	4 (13.3)	10 (33.3)	
c-ErbB2 overexpression*						NS
Absent	133	44 (33.0)	25 (18.7)	21 (15.7)	43 (32.3)	
Present	13	2 (15.3)	3 (23.0)	2 (15.3)	6 (46.1)	
E-cadherin expression†						NS
Normal	93	24 (25.8)	22 (23.6)	15 (16.1)	32 (34.4)	
Aberrant	55	23 (41.8)	8 (14.5)	8 (14.5)	16 (29.0)	
Total	150	47 (31.3)	30 (20.0)	23 (15.3)	50 (33.3)	

NS, not significant; *4 cases are missing, †2 cases are missing.

type and the hMLH1 or c-erbB2 expression.

Relation between the mucin phenotype or genetic alteration, and the prognosis

A MUC5AC expression and p53 overexpression were associated with poor patient's survival on univariate analysis as well as on multivariate analysis ($p<0.05$) (Fig. 2). Although each mucin phenotype was related with patient survival with borderline significance ($p=0.07$ and $p=0.009$ by univariate and multivariate analysis, respectively), the group with the gastric or mixed phenotype was more likely associated with poor patient's survival than was the group with the intestinal or unclassified phenotype by univariate and multivariate analyses ($p<0.05$) (Fig. 2). Lymph node metastasis and the pathologic stage were also related with patient survival.

DISCUSSION

GCs are histologically classified into two types, differentiated and undifferentiated, or intestinal and diffuse, according to the degree of glandular formation by the tumor cells. Intestinal type tumors have generally been considered to arise from gastric mucosa with intestinal metaplasia, and the diffuse type tumors arise from ordinary gastric mucosa without intestinal metaplasia.[3] However, mucin histochemical and immunohistochemical examinations have recently demonstrated that the gastric and intestinal phenotypic cell markers are widely expressed in GCs.[5] In this study, the great majority (about 85%) of the

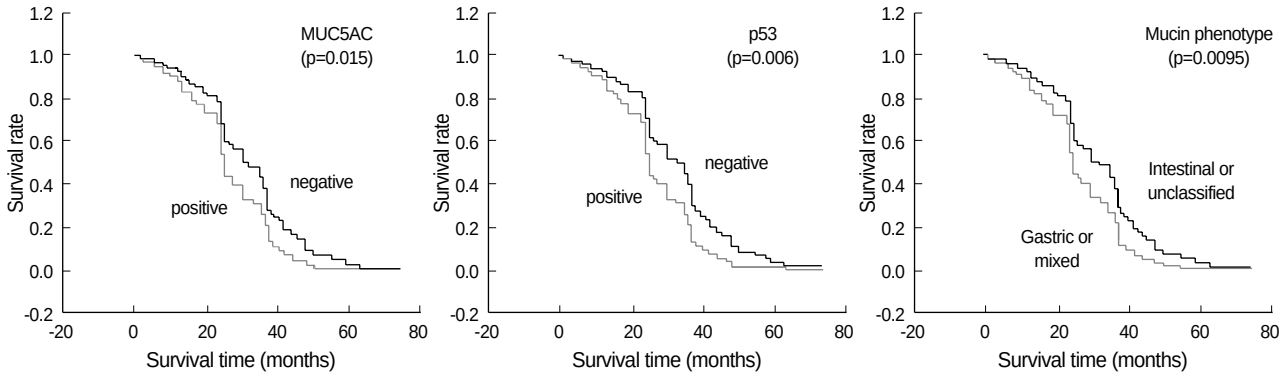

Fig. 2. Multivariate analysis by Cox proportional hazards model indicates that MUC5AC expression (A), p53 overexpression (B), and gastric or mixed phenotype (C) are associated with poor patient's survival.

intestinal histologic type cancers displayed variable degrees of a MUC1 or MUC5AC expression. We found no significant relationship between the histologic type and the mucin phenotype of the tumor. This result suggests that there is generally a free combination between the morphology and cellular mucin phenotypes.

MUC1 is a ubiquitous epithelial mucin. In the present study, a MUC1 expression was frequently observed in 63.3% of the cases. MUC1 was more frequently expressed in the intestinal histologic type (p=0.002). Tanaka *et al*.[28] reported that a normal E-cadherin/MUC1-negative expression pattern in gastric cancer is a favorable marker. They suggested that preoperative estimation of the E-cadherin status and MUC1 status of an endoscopic biopsy specimen might help select appropriate patients for minimally invasive treatment of gastric cancer. In the present study, patients with a normal E-cadherin/MUC1-negative expression pattern had a higher survival rate than those patients with other E-cadherin/MUC1 expression patterns, but the difference was not statistically significant (p=0.1, data not shown). Lee *et al*.[19] reported that patients with a MUC1-negative plus p53-negative expression pattern showed a better prognosis than the remaining cases. But in our series, the MUC1-negative plus p53-negative expression pattern was not correlated with patient survival (data not shown).

Several previous reports have indicated that MUC2 reactivity was associated with a favorable prognosis of intestinal-type GCs, and MUC1 reactivity was correlated with a poor prognosis.[18,19] In the current study, neither the MUC2 expression nor the MUC1 expression was associated with patient survival. , Reis *et al*.[16] and Machado *et al*.[13] reported that gastric-type differentiation was retained in the majority of GCs in early rather than advanced carcinoma. The current study also revealed that a MUC5AC expression was more likely associated with the early gastric cancer. But by survival analysis, a MUC5AC expression was considered as an independent indicator for a poor prognosis. This data suggests that the MUC5AC expression can be frequently retained in the early stage of the GCs and it can be related with a poor prognosis. In contrast to the result of this study, Jung *et al*.[30] reported that GCs with a MUC5AC expression had a better prognosis. We think that this difference seems to arise from the different statistical methods used in the two studies: univariate analysis versus multivariate analysis.

Ohmura *et al*.[4] and Endoh *et al*.[8] reported that each cellular phenotype followed a different genetic pathway, that is, foveolar-type tumors followed the mutator pathway that's characterized by microsatellite instability or mutation of MMR gene, and complete intestinal metaplastic phenotype tumors followed the "suppressor" pathway that's characterized by loss of heterozygosity of the tumor suppressor loci and also p53 overexpression. Further, ordinary-type tumors appeared to show mixed genetic alterations of both types. In the present study, the cases lacking a MLH1 expression revealed no definite relationship with the cellular mucin phenotype, but they were inversely associated with a MUC6 expression with borderline significance. p53 overexpression was correlated with the MUC1, MUC5AC and CD10 expressions. The incidence of p53 overexpression was higher in the gastric or mixed phenotype group than the intestinal or unclassified phenotype group. In addition, a p53 overexpression as well as the group with the gastric or mixed phenotype was associated with poor patient survival according to the survival analysis. This data suggests that the gastric or mixed phenotype may more likely go through the p53 pathway in carcinogenesis and is considered as an indicator for a poor prognosis.

The cell adhesion molecule E-cadherin forms a complex with catenins and it plays a key role in the construction of epithelial

tissue. Its down-regulation is potentially important in the metastases of carcinomas. Endoh *et al*.[11] detected an E-cadherin gene mutation in 21% of the differentiated type carcinomas of the gastric phenotype, although this mutation has generally been considered to be involved in undifferentiated types, but not in the differentiated type carcinomas. They speculated that the differentiated type carcinomas of the gastric phenotypes progressed to undifferentiated type carcinomas through the E-cadherin function and that the biological behavior of this type of tumor was aggressive. Zhou *et al*.[30] reported that a significant correlation exists between an E-cadherin expression and a poor survival rate. In this study, an aberrant E-cadherin expression was associated with the diffuse type by Lauren's classification ($p=0.002$), but not with the cellular mucin phenotypes or the survival rate.

Nakajima *et al*.[31] reported that overexpression and/or gene amplification of c-erb B2 might be poor prognostic factor in human GCs. However, in the current study, there was no relationship between c-erb B2 overexpression and patient survival. Nakajima *et al*.[31] also reported that overexpression and/or gene amplification of c-erb B2 occurred in intestinal type GCs. In the present study, although the incidence of c-erbB2 overexpression was higher in the unclassified phenotype than in any other of the mucin phenotypes, it did not reflect the histologic type according to the Lauren's classification.

In conclusion, this study infers a couple of points for GCs. First, the morphology is not consistent with the cellular mucin phenotype, and second, the gastric or mixed phenotype may more likely go through the p53 pathway in gastric carcinogenesis. Third, the mucin phenotype may be considered as a prognostic indicator.

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