Clinicopathologic Significance of Gastric and Intestinal Phenotypic Marker Expression in Gastric Carcinomas

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Background : It is well known that both gastric and intestinal phenotypic cell markers are expressed in gastric cancers. This study was aimed at investigating the correlation between gastric and intestinal phenotypic marker expression patterns of tumors and the clinicopathologic characteristics of gastric carcinomas.

Methods : We evaluated phenotypic marker expression by immunohistochemical staining with monoclonal antibodies. All tumors were classified as gastric (G), gastric and intestinal mixed (GI), intestinal (I), or null (N) phenotype.

Results : The tumors were phenotypically divided into G-phenotype tumors (33.2%), GI-phenotype tumors (25.7%), I-phenotype tumors (26.8%), and N-phenotype tumors (14.3%). N-phenotype tumors were associated with more corporeal location than GI- and I-phenotype tumors ($p=0.009$ and $p=0.007$, respectively), a larger size than I-phenotype tumors ($p=0.007$), a higher proportion of advanced gastric cancers than G-, GI-, and I-phenotype tumors ($p=0.003$, $p<0.001$, and $p<0.001$, respectively), more perineural invasion than G-, GI-, and I-phenotype tumors ($p=0.076$, $p=0.003$, and $p=0.003$, respectively), and more lymph node metastasis than GI-phenotype tumors ($p=0.017$). I-phenotype tumors were associated with a higher proportion of intestinal-type tumors than G-, GI-, and N-phenotype tumors ($p<0.001$, $p=0.011$, and $p<0.001$, respectively).

Conclusion : Our results indicate that the gastric and intestinal phenotypic marker expression pattern of tumors is prognostically useful for patients with gastric carcinoma.

Key Words : Gastric cancer; Immunohistochemistry; Phenotype; Prognosis

INTRODUCTION

Human gastric cancers may be histologically divided into two main groups, the intestinal type and the diffuse type, as proposed by Lauren's classification system[1]. As has been proposed by the Japanese Research Society for Gastric Cancer[2], these two types roughly correspond to the differentiated and undifferentiated types, respectively, according to the degree of glandular formation exhibited by the tumor cells. Although these classifications have been widely used, they are inadequate for research regarding the histogenesis of gastric

carcinomas and phenotype expression at the cellular level. This is largely due to the confusion of intestinal phenotypic cancer cells with "diffuse" structure and the presence of a gastric phenotype with the "intestinal" type of Lauren's classification[3]. 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. It has generally been believed that undifferentiated-type tumors arise from originary gastric mucosa without intestinal metaplasia. These two types of tumor are known to follow different genetic pathways during carcinogenesis[4, 5].

Table 1. Immunostains for the classification of gastric and intestinal mucin phenotype

Antigen (clone)	Cell phenotype	Source	Dilution
MUC5AC (CLH2)	Gastric foveoloar type	Novocastra Laboratories, Newcastle, UK	1:500
MUC6 (CLH5)	Gastric glandular type	Novocastra Laboratories, Newcastle, UK	1:500
MUC2 (Ccp58)	Goblet cell type	Novocastra Laboratories, Newcastle, UK	1:500
CD10 (56C6)	Small intestinal type	Novocastra Laboratories, Newcastle, UK	1:100

Analysis of the phenotypic expression of each gastric cancer cell is possible through the use of cell markers from the gastric and intestinal epithelia[6-12]. Independent of histological type, human gastric cancers at an early stage mainly consist of malignant cells of the gastric phenotype, while their advanced counterparts tend to have more malignant cells of the intestinal phenotype with progression[6, 11, 13, 14]. It has been reported that differentiated-type gastric carcinomas of the gastric phenotype (G-phenotype) are more likely to transform into undifferentiated-type carcinoma and show infiltrative growth into the deeper layers of the mucosa or invasion of the surrounding structures[9, 15, 16]. Eodoh et al.[15] detected an E-cadherin gene mutation in 21% (4/19) of the differentiated-type carcinomas of the G-phenotype, although this mutation has generally been considered to be involved in undifferentiated-type carcinomas, and not differentiated-type. The aforementioned study speculated that differentiated-type carcinomas of the G-phenotype progressed to undifferentiated-type carcinomas through the loss of the E-cadherin function and that the biological behavior of this type of tumor was particularly aggressive[15].

It is now clear that, in terms of the gastric and intestinal phenotypic marker expression pattern of the tumor, gastric carcinomas can be of various types[6, 7]. With respect to the clinicopathologic significance of the gastric and intestinal phenotypic marker expression pattern of gastric carcinomas, Koseki et al.[9] reported the G-phenotype to be an independent factor associated with lymph node metastasis among differentiated-type early gastric carcinomas. However, several authors[10, 17-22] have demonstrated a correlation between the prognosis and the phenotypic markers in gastric cancers. Still, concrete conclusions have yet to be drawn.

Therefore, in order to investigate the clinicopathologic significance of gastric and intestinal phenotypic marker expression patterns among the gastric carcinomas, we examined surgically resected tumor specimens from 343 patients with gastric carcinoma by immunohistochemical staining with monoclonal antibodies CLH2 (ant-MUC5AC), CLH5 (anti-MUC6), Ccp58 (anti-MUC2), and 56C6 (anti-CD10).

MATERIALS AND METHODS

Patients

We examined 343 primary gastric cancer specimens that had been surgically resected at Pusan National University Hospital, Pusan, Korea, from January 2002 and June 2004. The participants in this study included 235 men and 108 women, aged 58.0±11.2 years (mean±standard deviation). No preoperative radiotherapy or chemotherapy had been administered to these patients.

Clinicopathologic review

All specimens were fixed in 10% buffered formalin. Carcinomas with adjacent non-neoplastic mucosa were serially cut into 5-mm slices in parallel with the lesser curvature and embedded in paraffin. These were then sectioned and stained with hematoxylin-eosin for histological examination. Carcinomas were classified by Lauren's classification[1]. Clinicopathologic findings such as age, gender, tumor site, tumor size, histologic type, depth of invasion, lymphatic invasion, blood vessel invasion, perineural invasion, and lymph node metastasis were reviewed according to the Japanese Classification of Gastric Carcinomas[2].

Immunohistochemistry

We examined the expression of MUC5AC, MUC6, MUC2, and CD10 in carcinoma cells by immunohistochemistry (Table 1), as previously described[3, 9, 10, 23, 24]. Briefly, 4 m-thick consecutive sections were deparaffinized and hydrated through a graded series of alcohol. After inhibition of endogenous peroxidase activity by immersion in 3% H_2O_2/methanol solution, antigen retrieval was carried out with 10 mmol/l citrate buffer (pH 6.0) in a microwave oven for 10 minutes at 120℃. The sections were then incubated with the primary antibodies. Next, subsequent to thorough washing in phosphate-buffered solution (PBS), the sections were incubated first with biotinylated secondary antibody, and then with avidin-biotinylated horseradish peroxidase complex (Vectastain Elite ABC kit, Vector Laboratories30, Burlingame, Calif., USA). Finally, the immune complexes were visualized by incubation with 0.01% H_2O_2 and 0.05% 3,3-diaminobenzidine tetrachloride (DAB). Nuclear counterstaining was accomplished using Mayers hematoxylin.

The results of each antibody staining were evaluated with reference to the percentage of positively-stained cancer cells. A result was considered positive if at least 10% of the cells were stained. When less than 10% of cancer cells were stained, the
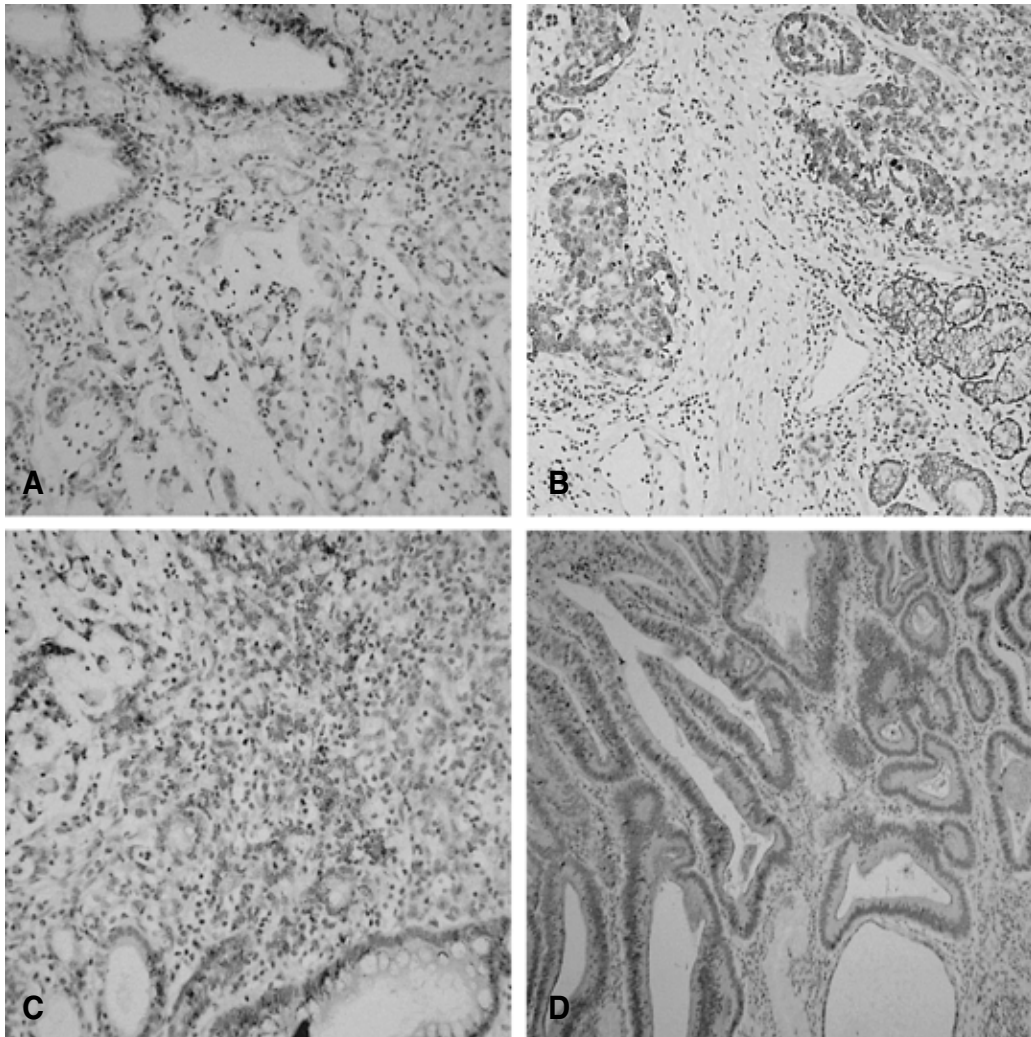
Figure 1. Gastric (A, B) and intestinal (C, D) phenotypic expression in gastric carcinomas. MUC5AC (A), MUC6 (B), MUC2 (C), and CD10 (D) are expressed in the cancer cells, respectively (×200).

results of immunostaining were considered negative.

Classification of mucin phenotype

MUC5AC and MUC6 are markers of gastric epithelial cells, whereas MUC2 and CD10 are typical of the intestinal epithelial cell phenotype[3, 9, 10, 25]. Gastric cancers in which more than 10% of the section area consisted of at least one gastric or intestinal epithelial cell phenotype were classified as G-phenotype or I-phenotype cancers, respectively. Those which exhibited both gastric and intestinal phenotypes were classified as gastric and intestinal mixed phenotype (GI-phenotype) cancers, while those showing neither gastric nor intestinal phenotype expression were grouped as cancers of the null phenotype (N-phenotype).

Statistical analysis

The data were analyzed for differences between the groups using Student's t-test, Fischers exact test, or x^2 test. Values of $p < 0.05$ were considered statistically significant. Statistical calculations were performed with SPSS version 10.0 for Windows software (SPSS Inc., Chicago, IL, USA).

RESULTS

Gastric and intestinal phenotypic marker expression in 343 gastric carcinomas

The expression of MUC5AC, MUC6, MUC2, and CD10 was demonstrated in 182 (53.1%), 60 (17.5%), 157 (45.8%), and 61 (17.8%) of the 343 gastric carcinomas, respectively (Figure 1). Taking into account the combinations of expression of these four markers, the 343 gastric carcinomas were respectively classified into G-phenotype tumors (114; 33.2%), GI-phenotype

Table 2. Correlations between clinicopathologic findings and expression of MUC5AC, MUC6, MUC2, and CD10 in 343 gastric carcinoma patients

Clinicopathologic findings	MUC5AC			MUC6			MUC2			CD10		
	(−) (n=161)	(+) (n=182)	p value	(−) (n=283)	(+) (n=60)	p value	(−) (n=186)	(+) (n=157)	p value	(−) (n=282)	(+) (n=61)	p value
Age, years	58.7 ±11.1	57.4 ±11.3	0.281	58.0 ±11.0	58.2 ±12.2	0.881	57.3 ±11.2	58.8 ±11.1	0.209	57.0 ±11.5	62.5 ±8.0	<0.001
Gender (M/F)	121/40	114/68	0.013	195/88	40/20	0.735	124/62	111/46	0.423	186/96	49/12	0.028
Tumor site												
Body	66	72	0.787	119	19	0.136	90	48	0.001	112	26	0.675
Antrum	95	110		164	41		96	109		170	35	
Tumor size												
<50 mm	109	124	0.932	193	40	0.817	120	113	0.140	185	48	0.047
≥50 mm	52	58		90	20		66	44		97	13	
Histology type												
Intestinal	110	89	0.001	159	40	0.059	97	102	0.019	146	53	<0.001
Diffuse	45	87		116	16		84	48		127	5	
Mixed	6	6		8	4		5	7		9	3	
Depth of invasion												
EGC	71	92	0.233	131	32	0.321	74	89	0.002	130	33	0.257
AGC	90	90		152	28		112	68		152	28	
Vascular invasion												
(−)	105	126	0.429	187	44	0.276	123	108	0.601	193	38	0.353
(+)	56	56		96	16		63	49		89	23	
Lymphatic invasion												
(−)	96	124	0.101	179	41	0.456	115	105	0.331	182	38	0.740
(+)	65	58		104	19		71	52		100	23	
Perineural invasion												
(−)	109	128	0.599	192	45	0.276	117	120	0.007	192	45	0.384
(+)	52	54		91	15		69	37		90	16	
Lymph node metastasis												
(−)	91	109	0.528	165	35	0.997	99	101	0.038	162	38	0.486
(+)	70	73		118	25		87	56		120	23	

tumors (88; 25.7%), I-phenotype tumors (92; 26.8%), and N-phenotype tumors (49; 14.3%).

Correlations between clinicopathologic findings and MUC5AC, MUC6, MUC2, and CD10 expression in 343 gastric carcinomas

The correlations between the clinicopathologic findings and the expression of MUC5AC, MUC6, MUC2, and CD10 in the 343 gastric carcinoma patients are shown in Table 2. MUC5AC expression was associated with a lower male-to-female ratio and a higher proportion of diffuse-type tumors (p=0.013 and p=0.001, respectively). No significant correlations were observed between MUC6 expression and any of the variables examined. MUC2 expression was correlated with a higher proportion of antral location, intestinal-type tumors, and early gastric cancers (p=0.001, p=0.019, and p=0.002, respectively). It was also associated with lower proportions of perineural invasion and lymph node metastasis (p=0.007 and p=0.038, respectively). CD10 expression was correlated with a higher mean patient age, a higher male-to-female ratio, and a higher proportion of

intestinal-type tumors (p<0.001, p=0.028, and p<0.001, respectively).

Correlations between clinicopathologic findings and phenotypic marker expression patterns in 343 gastric carcinomas

The correlations between the clinicopathologic findings and the phenotypic marker expression pattern of the tumors of 343 gastric carcinoma patients are shown in Table 3. G-phenotype tumors were associated with a lower male-to-female ratio than GI-, I-, and N-phenotype tumors (p=0.066, p=0.010, and p=0.017, respectively). N-phenotype tumors were associated with more corporeal location than were GI- and I-phenotype tumors (p=0.009 and p=0.007, respectively), a larger size than I-phenotype tumors (p=0.007), a higher proportion of advanced gastric cancers than G-, GI-, and I-phenotype tumors (p=0.003, p<0.001, and p<0.001, respectively), more perineural invasion than G-, GI-, and I-phenotype tumors (p=0.076, p=0.003, and p=0.003, respectively), and more lymph node metastasis than GI-phenotype tumors (p=0.017). I-phenotype tumors were

Table 3. Correlations between clinicopathologic findings and phenotypic marker expression patterns of the tumors of 343 gastric carcinoma patients

Clinicopathologic findings	Phenotypic expression of the tumor			
	G phenotype (n=114)	GI phenotype (n=88)	I phenotype (n=92)	N phenotype (n=49)
Age, years	57.5±11.6	57.8±10.9	60.1±10.5	55.6±11.7
Gender (M/F)	66/48[a]	62/26	69/23	38/11
Tumor site				
Body	49	30	31	28
Antrum	65	58	61	21[b]
Tumor size				
<50 mm	74	61	71	27[c]
≥50 mm	40	27	21	22
Histology type				
Intestinal	54	52	72d	21
Diffuse	55	33	17	27
Mixed	5	3	3	1
Depth of invasion				
EGC	54	49	49	11[e]
AGC	60	39	43	38
Vascular invasion				
(−)	76	63	61	31
(+)	38	25	31	18
Lymphatic invasion				
(−)	72	63	56	29
(+)	42	25	36	20
Perineural invasion				
(−)	75	67	70	25[f]
(+)	39	21	22	24
Lymph node metastasis				
(−)	63	58	57	22[g]
(+)	51	30	35	27

(−), negative; (+), positive; EGC, early gastric cancer; AGC, advanced gastric cancer
[a]$p<0.05$ compared to the I, N phenotype
[b]$p<0.01$ compared to the GI, I phenotype
[c]$p<0.01$ compared to the I phenotype
[d]$p<0.001$ compared to the G, GI, N phenotype
[e]$p<0.01$ compared to the G, GI, I phenotype
[f]$p<0.01$ compared to the GI, I phenotype
[g]$p<0.05$ compared to the GI phenotype

associated with a higher proportion of intestinal-type tumors than G-, GI-, and N-phenotype tumors ($p<0.001$, $p=0.011$, and $p<0.001$, respectively).

DISCUSSION

In the present study, 343 gastric carcinomas were classified as G-phenotype tumors (114; 33.2%), GI-phenotype tumors (88; 25.7%), I-phenotype tumors (92; 26.8%), and N-phenotype tumors (49; 14.3%). Previous reports have shown the incidences of G-, GI-, I-, and N-phenotype tumors to be 17.7~41.1%, 20.3~60.1%, 18.5~46.6%, and 0~31.6%, respectively[10, 15, 22, 26, 27]. These data are consistent with our results. These results indicate that gastric carcinomas can express both the G-phenotype and the I-phenotype to varying degrees.

Here, we showed the expression of MUC5AC, MUC2C, and CD10 demonstrated in 182 (53.1%), 157 (45.8%), and 61 (17.8%) of the 343 gastric carcinomas, results which are compatible with previous studies[10, 19, 22, 28]. The MUC6 expression (60/343, 17.5%) in the present study differed from the results of the study by Tajima et al.[10], who found MUC6 expression in 87 (64%) of 136 gastric carcinomas. However, our data was in agreement with several other reports which found that MUC6 immuno-expression was present in about 13~37% of gastric cancers, and these previous reports demonstrated this using the same anti-MUC6 monoclonal antibody[19, 22, 25, 28, 29]. Therefore, we must consider the possibility that the rate for MUC6 expression might be linked with the predomination of the G- and GI-phenotypes (36.8% and 41.2%, respectively), and the consequent lower

proportion of I- and N-phenotypes (15.4% and 6.6%, respectively), as reported by Tajima et al.[10]. In regards to the correlations between the clinicopathologic findings and MUC2 expression, some reports have shown no significant correlation between the two[10, 28, 30]. However, Utsunomiya et al.[31] reported that MUC2 expression by the tumor was associated with lower levels of invasion and lymph node metastasis. We also found that MUC2 expression was associated with early gastric carcinoma and a lower proportion of lymph node metastasis. The reason for the discrepancies between the results of the aforementioned study and our results may be that the patients in most of the previous studies had advanced gastric carcinoma, whereas our study, and that of Utsunomiya et al., consisted of patients with both early and advanced gastric carcinoma. Additionally, we found that MUC2 expression correlated with a higher proportion of antral location and intestinal-type tumors, and a lower proportion of perineural invasion. In regards to the correlations between the clinicopathologic findings and CD10 expression, CD10 expression was reported to be correlated with a higher rate of differentiated-type tumors[10, 32] and a higher male-to-female ratio[10]. We also found that CD10 expression was associated with a higher male-to-female ratio, a higher proportion of intestinal-type tumors, and a higher mean patient age.

MUC5AC expression was previously reported to have no association with any clinicopathological features[19, 20]. It was, however, reported to have an association with early gastric carcinoma[33] and diffuse-type carcinoma[28, 33]. In the present study, MUC5AC expression correlated with a higher proportion of diffuse-type tumors and a lower male-to-female ratio. MUC6 expression was previously reported to have no association with any clinicopathologic features[10, 19, 28]. We also found no correlations between MUC6 expression and any of the variables examined.

In the previous studies, no correlation was reported between the phenotypic marker expression pattern of the tumor and gender[9, 10, 22]. Yet, in the present study, G-phenotype tumors were associated with a lower male-to-female ratio than the other types of tumor. Tajima et al.[10] reported that I-phenotype tumors were associated with a higher rate of differentiated-type tumors than G-phenotype tumors. Similarly, in the present study, the I-phenotype tumors were associated with a higher proportion of intestinal-type tumors than G-, GI-, and N-phenotype tumors. Tajima et al.[10] have previously shown that G-phenotype tumors were associated with a significantly poorer outcome than I-phenotype tumors. Mizoshita et al.[22] reported that the 5-year survival rates forpatients with G-, GI-, I-, and N-phenotype gastric cancers were 37.5%, 72.2%, 54.7%, and 33.9%, respectively. They also showed that patients with GI-phenotype cancers had the best outcome and those with

N-phenotype had the worst outcome. Additionally, they reported that the phenotypic classification was an independent prognostic factor, together with depth of invasion and lymph node metastasis. Our results similarly showed that N-phenotype tumors were associated with a higher proportion of advanced gastric cancers and a higher proportion of perineural invasion than tumors of other phenotypes. Moreover, N-phenotype tumors were also associated with more lymph node metastasis than GI-phenotype tumors. These results may be partially explained by the fact that the phenotypic expression of mucin may have changed during the course of the disease, especially in the case of larger tumors. These later-stage cancer lesions may lose the mucin phenotype that had been expressed at an earlier stage.

In conclusion, we have shown that N-phenotype tumors were associated with a higher proportion of advanced gastric cancer and a higher proportion of perineural invasion than tumors of other phenotypes. These results indicate that evaluating the phenotypic marker expression pattern, as determined by the combination of MUC5AC, MUC6, MUC2, and CD10 expression, is prognostically useful for patients with gastric carcinoma.

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