Histol Histopathol (2014) 29: 343-351
DOI: 10.14670/HH-29.343

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

The relationship between mucin phenotype and clinicopathological factors, proliferative activity and p53 expression in gastric hyperplastic polyps

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Summary. Aims: Gastric hyperplastic polyps (GHPs) are the most common polypoid lesion of the stomach, and their malignant potential has been demonstrated. In the present study, we evaluated the mucin phenotypes of GHPs and investigated the relationships among mucin phenotypes and clinical-pathological factors, proliferative activity and p53 expression in GHPs. Methods: The CD10, MUC2, MUC5AC and MUC6 expression patterns of 238 GHPs were examined by immunohistochemical staining. The GHP mucin phenotypes were divided into 4 subtypes: the gastric mucin phenotype (G-type), the intestinal mucin phenotype (I-type), the mixed or gastrointestinal mucin phenotype (GI-type) and the unclassified mucin phenotype (U-type). Results: The G and GI types were observed in 58% and 42% of the GHPs, respectively. However, no I or U type GHPs were found in the present study. The GI type was more common in lesions with dysplasia or carcinoma than in polyps without dysplasia or carcinoma ($P<0.001$). P53-positivity rate and high index of Ki-67 tumors were significantly more common in the GI-type than in the G-type polyps ($P<0.001$). Conclusions: The mucin phenotype may serve as a useful marker for the malignant potential of GHPs, and GI-type GHPs should be considered to be a lesion with malignant potential.

Key words: Gastric hyperplastic polyps, Mucin phenotypes, Proliferative activity, p53

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Introduction

Gastric hyperplastic polyps (GHPs) are the most common polypoid lesions of the stomach. The risk of malignant GHP transformations was once thought to be low, and GHPs were thought to be benign lesions. However, there have been a number of recent reports suggesting that GHPs can exhibit malignant potential. The incidence of carcinomatous GHPs conversion ranges from 0.3% to 7.1% (Orlowska et al., 1995; Zea-Iriarte et al., 1996; Murakami et al., 2001; Han et al., 2009; Richa, 2009).

Several molecular alterations have been described in GHPs. Some studies have demonstrated that p53 overexpression (Lauwers et al., 1993; Zea-Iriarte et al. 1996; Murakami et al., 2001; Yao et al., 2002), elevated Ki-67 labeling indices (Zea-Iriarte et al. 1996; Murakami et al., 2001; Yao et al., 2002) and cyclin D1 overexpression (Murakami et al., 2001) in the area of dysplasia or carcinoma in GHPs compared with normal and benign hyperplastic gastric mucosa. Microsatellite instability (MSI) (Nogueira et al., 1999) and loss of heterozygosity (LOH) (Lauwers et al., 1993; Dijkhuizen et al., 1997) have also been described in gastric hyperplastic polyps (GHPs), they could not well documented that GHPs would undergo malignant transformation. P53 (Dijkhuizen et al., 1997; Murakami et al., 2001) and K-ras mutations (Murakami et al., 2001) were also studied in GHPs with/without dysplasia or carcinoma, but with conflicting results.

Recently, some studies have focused on mucin phenotypes by examining the role of MUC2, MUC5AC, MUC6 and CD10 in gastric cancer. Many studies have

shown that the mucin phenotype is associated with the clinicopathological characteristics and prognosis of gastric carcinoma, suggesting that the mucin phenotype may be a useful prognostic marker for gastric carcinoma (Shiroshita et al., 2004; Sugai et al., 2004a,b, 2010; Lee et al., 2009a; Takano et al., 2009). However, the significance of the mucin phenotype for GHPs has not been well documented.

Mucins are large molecular weight glycoproteins that are expressed by various epithelial cells. Mucins can be categorized into two main subgroups according to their physiological characteristics: "secreted/gel-forming" mucin and "membrane-bound" mucin. MUC2 is characteristically expressed in intestinal (Toribara et al., 1991) and colorectal epithelium (Escande et al. 2004) goblet cells. MUC5AC is primarily expressed in gastric epithelial cells (Brockhausen, 1999). MUC6 is expressed in mucosa neck cells and in pyloric gland cells of the stomach (Bartman et al., 1998).

CD10, also known as Common Acute Lymphocytic Leukemia Antigen (CALLA), is a cell surface enzyme with neutral metalloendopeptidase activity (Maguer-Satta et al., 2011). CD10 is expressed on the cells of lymphoblastic, follicular germinal center lymphomas and on cells from patients with chronic myelocytic leukemia (CML) (Maguer-Satta et al., 2011). CD10 is also expressed on the brush border of gut epithelial cells (Maguer-Satta et al., 2011). P53 is a tumor suppressor gene. P53 gene mutations are known to play important roles in the carcinogenesis of many human tumors, including colonic carcinoma and gastric carcinoma (Wang et al., 2012). Ki-67 is a nuclear protein that is expressed in proliferating cells. Ki-67 is preferentially expressed during late the G1, S, M and G2 phases of the cell cycle, while cells in G0 are quiescent for this protein (Scholzen and Gerdes, 2000).

In the present study, we evaluate the mucin phenotypes in GHPs to elucidate the relationships among mucin phenotypes and clinicopathological factors, proliferative activity and p53 expression in GHPs, and to provide insight into the malignant potential of GHPs.

Materials and methods

Samples

Two hundred thirty-eight (238) GHPs/patients who underwent endoscopic polypectomy or gastrectomy were retrieved from the records of the Department of Pathology, the Second Affiliated Clinical College of Harbin Medical University, China, between January 2005 and April 2011. Thirty samples of normal gastric mucosa were obtained from the gastrectomy material around the gastric carcinoma and were used as controls. The normal mucosa was sampled from a proximal surgical margin that was greater than 5 cm from the carcinoma (Jiao et al., 2008). In this study, the original hematoxylin and eosin sections were reviewed by two GI pathologists (YF J, LH W), and the specimens that were inadequate for a convincing diagnosis were not included. The following pathological diagnostic criteria were used for GHP: irregular, cystically dilated, and elongated foveolar epithelium or foveolar epithelium with architecturally distorted glands; and stroma with inflammation, edema, reactive mesenchymal cells and smooth muscle hyperplasia (Richa, 2009). All of the patients in the present study were negative for family history and clinical manifestations of Peutz-Jeghers syndrome there being close histological similarities between Peutz-Jeghers polyps and GHP. The maximum diameter of each polyp was recorded. The locations of the GHPs were classified as the upper (U), middle (M) and lower (L) third of the stomach. The upper (U) third corresponded to the cardia or fundus, the middle (M) third corresponded to the corpus; and the lower (L) third corresponded to the antrum and pylorus. All of the procedures were consistent with the university's ethical standards (approval No. H13-9) and hospital criteria. All of the participants provided informed consent.

Immunohistochemical staining

The immunohistochemical staining was performed on 3-μm-thick formalin-fixed, paraffin-embedded tissue sections. The antibodies, their source and the dilution used in this study are shown in Table 1. Briefly, after the sections were deparaffinized and dehydrated with xylene and ethanol, they were treated with Thermofisher peroxidase-blocking solution for 10 min at room temperature to block the endogenous peroxidase activity. The antigen retrieval was performed using an autoclave under variable conditions (Table 1). The sections were incubated with a primary antibody using variable times and conditions (Table 1). The EnVision™ detection reagent (Thermofisher) was used as a secondary

Table 1. Detailed primary antibodies information and dilutions.

Antibody	Clone	Dilution	Antigen retrieval	Incubation	Source
CD10	56C6	1:50	EDTA	4°C, overnight	Thermofisher
MUC2	M53	1:500	citrate buffer	4°C, overnight	Thermofisher
MUC5AC	1-13M1	1:1000	citrate buffer	4°C, overnight	Thermofisher
MUC6	CLH5	1:50	citrate buffer	4°C, overnight	Thermofisher
Ki-67	MIB-1	1:200	citrate buffer	4°C, overnight	Thermofisher
P53	DO-7	1:100	citrate buffer	4°C, overnight	Thermofisher

antibody. 3'3-diaminobenzidine was used as a chromogen. Hematoxylin was used as the counter stain. The appropriate positive and negative (primary antibody replaced by normal immunoglobulin) controls were processed simultaneously. All of the immunohistochemical staining was performed at least twice for each sample. All of the cases included adequate positive controls, negative controls and internal controls.

Evaluation and classification of the GHP mucin phenotypes

The polyps were scored as either negative or positive for CD10 and each of the mucin antibodies. Positive expression was defined by the presence of cytoplasmic and/or membranous reactivity in the lesional epithelium. The CD10, MUC2, MUC5AC and MUC6 positivities were deemed significant when >5% of cells were positive. The mucin phenotypes were classified according to their combined expression (Table 2): a gastric phenotype (G type) was defined as a GHP with predominantly MUC5AC and/or MUC6 intracytoplasmic mucin but with no MUC2- and CD10- positive cells [MUC5AC or MUC 6 (+) and CD10 combined with MUC2 (-)]; an intestinal mucin phenotype (I-type) was defined as a GHP with MUC2-positive cells and/or CD10-positive cells [CD10 and/or MUC2(+) and MUC5AC combined with MUC6 (-)]; a mixed or gastrointestinal mucin phenotype (GI type) was defined as a GHP having immunostaining consistent with both gastric (MUC5AC and/or MUC6 positive) and intestinal (MUC2 and/or CD10 positive) phenotypes [MUC5AC and/or MUC 6 (+) and CD10 or/and MUC2 (+)]; and a GHP without immunostaining for either gastric or intestinal phenotypes were assigned to an unclassified type (U type) [MUC5AC combined with MUC 6 (-) and CD10 combined with MUC2 (-)] (Yao et al., 2002; Wakatsuki et al., 2008; Namikawa and Hanazaki, 2010).

The Ki-67 and p53 staining were determined to be either positive or negative on the basis of the presence or absence of nuclear staining; the number of positive cells per 1,000 cells was counted at x400 magnification in at least 5 random areas from each GHP zone (for a total of 5,000 cells counted). In the GHPs with LGD, HGD or Ca, the Ki-67 positive rate was calculated as the percentage of Ki-67-positive cell nuclei in 5 high-power fields (HPF, x400) from the LGD, HGD and Ca zones. The results are expressed as median percentages with ranges.

Statistical analysis

The statistical calculations were performed using the SPSS 13.0 software package (SPSS Inc., China). The clinical and pathological differences between the study and control groups were evaluated using the Mann-Whitney two-sided U test for the continuous data and using the χ^2 test for the categorical data (the Fisher exact test was used if the number of observations was <5). All of the significance tests were two-sided, and differences were considered statistically significant when P-values <0.05.

Results

The clinicopathological factors and histological findings

The clinicopathologic GHP findings used in this study are shown in Table 3. Of 238 GHPs, 35 (14.8%) were considered low-grade dysplasia (LGD), 5 (2.1%) were considered high-grade dysplasia (HGD), and 10 (4.2%) were considered well-differentiated adenocarcinomas. Dysplastic or cancerous GHPs were significantly (P=0.003) more common in the older patients than in the younger population. With respect to histological grading, 38% (19/50) of the dysplastic or cancerous GHPs were larger than 2 cm (P<0.001). In addition, HGD and carcinoma were predominantly found mostly in the pedunculated or semi-pedunculated GHPs (P=0.002). However, there was no significant gender or location difference. The clinical and histopathological data for all of the patients used in this study are summarized in Table 3.

GHP mucin phenotypes

The relationships among GHP mucin phenotypes and clinicopathological factors in 238 patients are also shown in table 3. One hundred thirty-eight (138) of the 238 GHPs (58%) were G-type, and 100 of the 238 polyps (42%) were GI-type (Figs. 1, 2). No I-type or U-type GHPs were found in this study. In GHPs without dysplasia or carcinoma (n=188), 56 of them were GI-type GHPs (29.8%). In 35 GHPs with LGD, 29 polyps were GI-typed (82.9%). All 15 GHPs with HGD or carcinoma showed the GI phenotype (100%). In contrast, LGD was found in only 6 of the 138 G-type GHPs (4.3%, P<0.001).

Ki-67 and p53 expressions

Ki-67 labeling index was greater in the GI-type (mean 47.5%) than in the G-type (mean 25.0%, P<0.001) GHPs (Fig. 3A). The Ki-67 labeling index was significantly greater in the dysplastic (mean 52.5%) than in the non-dysplastic (mean 29%) GHPs (P<0.001). In addition, the Ki-67 index gradually increased from GHP without dysplasia (mean 29%) to GHP with LGD (mean

Table 2. Classification of the GHP mucin phenotypes.

	MUC5AC or MUC6 +	MUC5AC and MUC6 -
CD10 and/or MUC2(+)	GI-type	I-type
CD10 and/or MUC2(-)	G-type	U-type

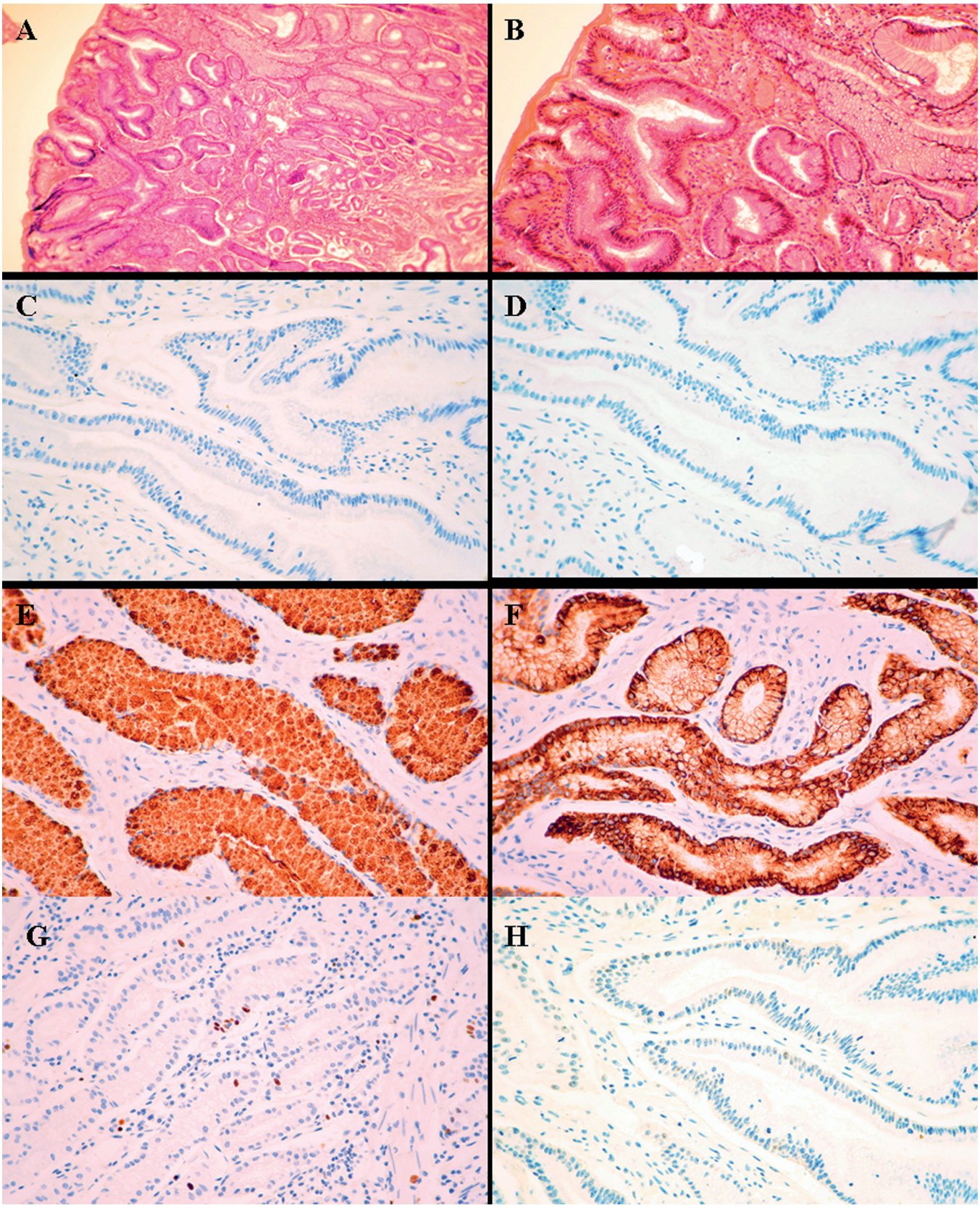

Fig. 1. The immunohistochemical staining pattern of a G-type GHP without dysplasia. **A and B** show hematoxylin and eosin staining of a GHP without dysplasia or carcinoma. The epithelial cells are negative for CD10 **(C)** and MUC2 **(D)**, but show immunoreactivity to MUC5AC **(E)** and MUC6 **(F).** The Ki-67 labeling of the lesion is low **(G)**. The p53 expression in the GHP is negative **(H)**. x 400

Table 3. The clinicopathological factors, histopathological types and mucin phenotypes of the GHPs.

	GHP without Dysplasia	GHP with LGD	GHP with HGD or Ca	P1 Value	G type	GI type	P2 Value
Gender	188 (79.0)	35 (14.7)	15 (6.3)	0.17	138 (58.0)	100 (42.0)	0.024
Males, %	103 (54.8)	23 (65.7)	11 (73.3)		71 (51.4)	66 (66.0)	
Females, %	85 (45.2)	12 (34.3)	4 (26.7)		67 (48.6)	34 (34.0)	
Age, years (mean±SE)	51.1±0.9	57.2±1.9	63.8 ±5.7	0.003	49.5±1.2	56.5±1.0	<0.001
Location				0.332			0.258
U, %	43 (22.9)	3 (8.6)	4 (26.7)		34 (24.6)	16 (16.0)	
M, %	31 (16.5)	6 (17.1)	2 (13.3)		23 (16.7)	15 (15.0)	
L, %	114 (60.6)	26 (74.3)	9 (60.0)		81 (58.7)	69 (69.0)	
Size, cm (%)				<0.001			0.091
<2	164 (87.2)	28 (80.0)	3 (20.0)		118 (85.5)	76 (76.0)	
≥2	24 (12.8)	7 (20.0)	12 (80.0)		20 (14.5)	24 (24.0)	
Gross appearance shape, %				0.002			0.347
Sessile	118 (62.8)	25 (71.4)	2 (13.3)		74 (53.6)	59 (59.0)	
Pedunculated	25 (13.3)	6 (17.1)	6 (40.0)		24 (17.4)	19 (19.0)	
Semi-pedunculated	45 (23.9)	4 (11.4)	7 (46.7)		40 (29.0)	22 (22.0)	
Pathologic grading (%)							<0.001
GHP					132 (95.7)	56 (56.0)	
GHP withLGD					6 (4.3)	29 (29.0)	
GHP with HGD or Ca					0 (0)	15 (15.0)	
Ki-67, %	29 (1-80)	47.8 (0-78)	55.9 (14-74)	<0.001	25 (1-80)	47.5 (1-78)	<0.001
P53, %	3 (0-60)	27.4 (0-78)	53.6 (35-87)	<0.001	6.9 (0-61)	29.5 (0-87)	<0.001

*SE, Standard error; LGD, Low grade dysplasia; HGD, High grade dysplasia; Ca, Carcinoma; The results of Ki-67 and p53 are expressed as median percentage with ranges; P1 The value expresses the significance of the GHP about histological grading; P2 The value expresses the significance of the GHP mucin phenotype.

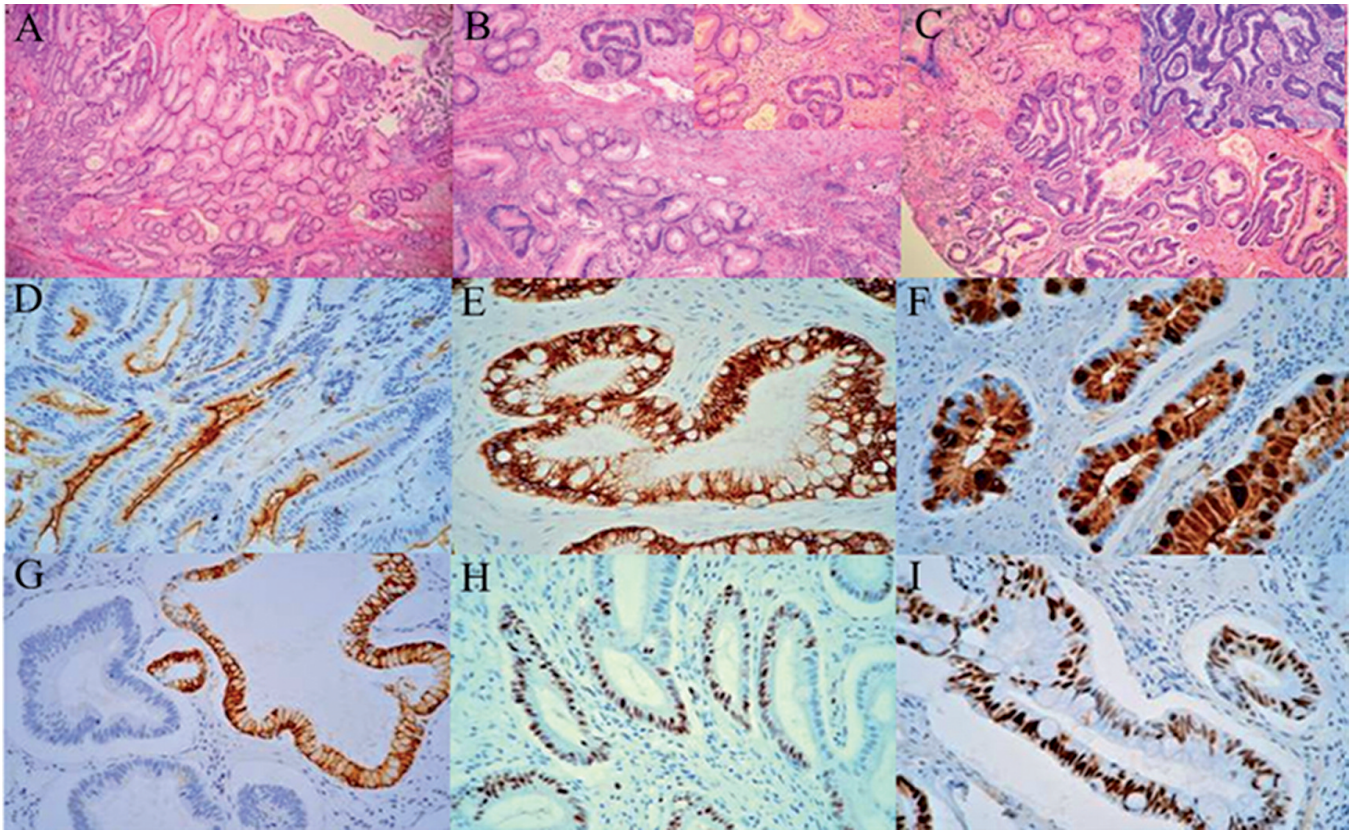

Fig. 2. The immunohistochemical staining pattern of a cancerous GI-type GHP. **A-C** show hematoxylin and eosin staining of a GHP with cancer. The epithelial cells are positive for CD10 **(D)**, MUC2 **(E)**, MUC5AC **(F)** and MUC6 **(G)**, with increased Ki-67 labeling **(H)**. There is strong p53 expression in the cancerous GHP **(I)**. x 400

47%), to GHP with HGD (mean 64%) and to GHP with carcinoma (mean 56%) (P<0.001) (Fig. 3B).

P53 overexpression was found more frequently in the GI-type (mean 29.5%) than in the G-type (mean 6.9%, P<0.001) GHPs (Fig. 3C). P53 expression was significantly greater in the dysplastic (mean 33%) than in the non-dysplastic (mean 3%) GHPs (P<0.001). Likewise, the p53 expression rates gradually increased from GHP without dysplasia (mean 3%) to GHP with LGD (mean 28%), to GHP with HGD (mean 43%) and to GHP with carcinoma (mean 56%) (P<0.001) (Fig. 3D).

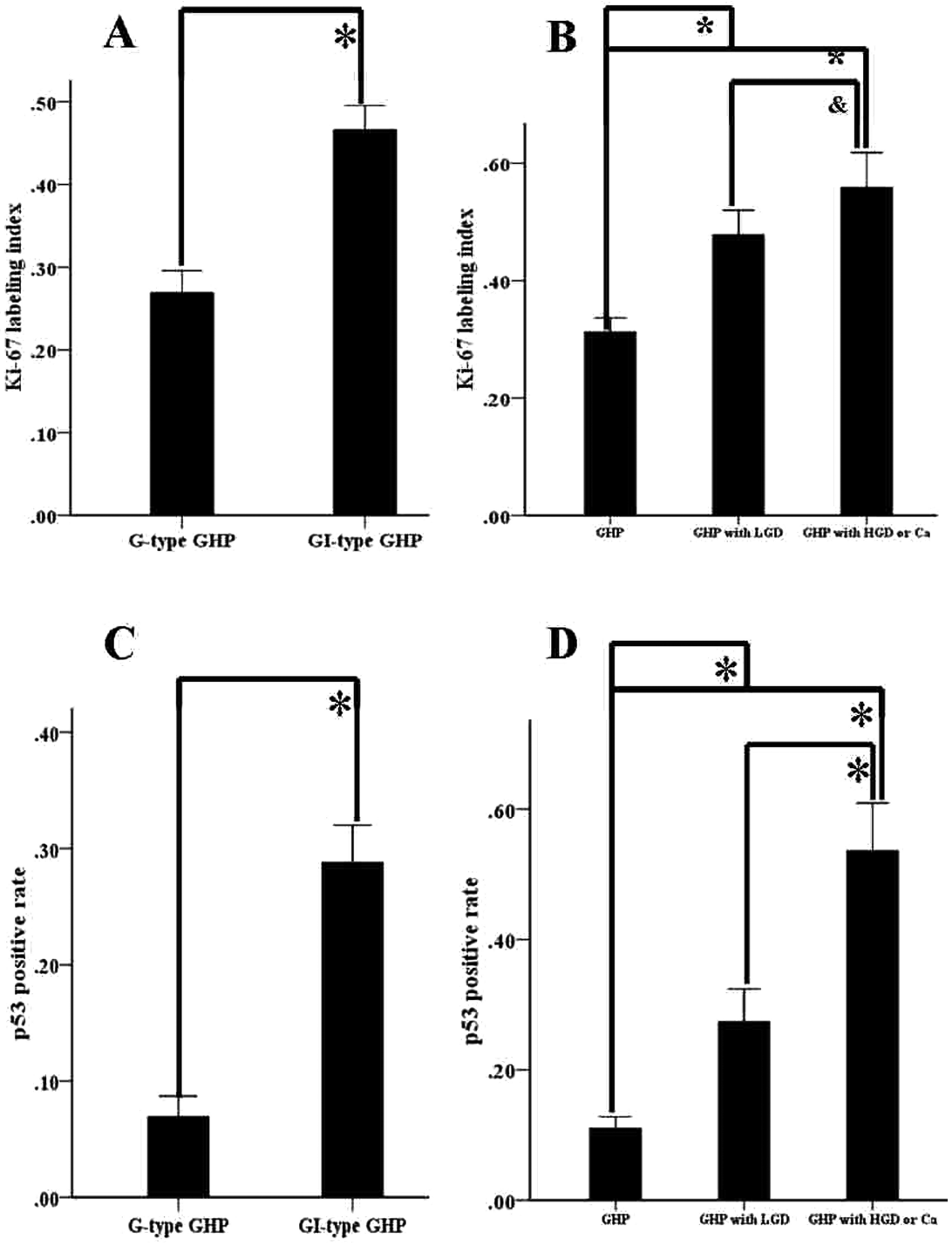

Fig. 3. A. Comparison of the Ki-67 labeling index between the G and GI types. Ki-67 expression was greater in the GI-type (47.5%, 1-78%) than in the G-type (25.0%, 1-80%, P<0.001) of GHPs. **B.** Comparison of the p53-positivity between the G and GI types. P53 overexpression was found more frequently in the GI-type (29.5%, 0-87%) than in the G-type (6.9%, 0-61%). **C.** Comparisons of the Ki-67 labeling index among GHP, GHP with LGD, GHP with HGD and GHP with Ca. The rates of Ki-67 expression were significantly greater in the dysplastic (52.5%, 8-78%) than in the non-dysplastic (29%, 1-80%) GHPs (P<0.001). The Ki-67 expression rates gradually increased from GHP without dysplasia (29%, 1-80%) to GHP with LGD (47%, 8-78%), to GHP with HGD (64%, 59-74%) and to GHP with carcinoma (56%, 14-67%) (P>0.001). **D.** Comparisons of the p53-positive rate among GHP, GHP with LGD, GHP with HGD and GHP with Ca. The rates of p53 expression were significantly greater in the dysplastic (33%, 0-87%) than in the non-dysplastic (3%, 0-60%) GHPs (P<0.001). The p53 expression rates gradually increased from GHP without dysplasia (3%, 0-60%) to GHP with LGD (28%, 0-78%), to GHP with HGD (43%, 35-76%) and to GHP with carcinoma (56%, 38-87%) (P<0.001) *P<0.0001; &P<0.05.

The relationship between mucin phenotype and clinicopathological factors

In the older patients, the GI type was more common than the G type (P<0.001). The number of patients older than 50 years with GI-type GHPs was significantly greater than the number of patients with G-type GHPs (P<0.001). The number of male patients with GI-type GHPs was greater than the number of female patients with G-type GHPs (P=0.024). The fraction of GI-type GHPs that were larger than 2 cm (24 of 100, 24%) was greater than that of the G-type GHPs (20 of 138, 14.5%), although the sizes of the G- and GI-type GHPs were not significantly different (P=0.091). There were no statistically significant relationships between the two phenotypes in location, gross appearance or shape.

Discussion

This study is the first to report the relationships among mucin phenotypes and clinicopathological factors, proliferative activity and p53 expression in a large GHP series. Interestingly, only the G- and GI-types were found in our study. Our data showed that all of the GHPs with HGD or carcinoma and most of the GHPs with LGD were of the GI-type. By contrast, most GHPs without dysplasia were of the G-type. In addition, we observed a higher p53 expression rate and Ki-67 index in the GI-type than in the G-type GHPs (P<0.001). Taken together, our results suggest that GI-type (rather than G-type) GHPs have malignant potential. The GHPs with dysplasia or carcinoma were predominantly found in the older patients in this study (P=0.003), a result that is consistent with the findings of Dirschmid (Dirschmid et al., 2006). We also observed that the Ki-67 index and p53 expression gradually increased with increasing dysplasia (P?0.001), results which were similar to those of previous studies (Lauwers et al., 1993; Zea-Iriarte et al., 1996; Murakami et al., 2001; Yao et al., 2002). In the present study, the GI-type GHPs were predominantly found in the older patients. The number of patients older than 50 years with GI-type GHPs was significantly greater than the number of patients with G-type GHPs (P<0.001). Moreover, the number of male patients with GI-type GHP was greater than the number with G-type GHPs (P=0.024). These results indicate that patients older than 50 years (especially the male patients) with GI-type GHPs should be considered at risk for gastric cancer. Previous studies have shown that larger GHPs are more likely to undergo malignant transformation and have suggested that GHPs larger than 1 cm should be removed by either endoscopic or surgical resection (Lauwers et al., 1993; Scholzen and Gerden, 2000; Yao et al., 2002; Lee et al., 2009b). In our study, most GHPs with HGD or carcinoma were larger than 2 cm, similar to other studies (Orlowska et al., 1995; Zea-Iriarte et al., 1996; Murakami et al., 2001; Han et al., 2009; Richa 2009). The rate of malignant transformation was 4.2% in our series.

Similar to other studies (Daibo et al., 1987; Zea-Iriarte et al., 1996; Han et al., 2009), all of the cancerous GHPs in the present study were well-differentiated (intestinal type) gastric adenocarcinomas. It is well known that incomplete intestinal metaplasia is more likely to cause gastric cancer than complete intestinal metaplasia (Gomyo et al., 1996; Hamamoto et al., 1997; Driessen et al., 1998; Cassaro et al., 2000; Weiss et al., 2003). Some authors have also classified intestinal metaplasia into a complete intestinal mucin phenotype (Comp I type) [CD10 (+) and MUC5AC combined with MUC6 (-)] and incomplete intestinal mucin phenotype (Incomp I type) [MUC5AC or MUC 6 (+) and CD10 or MUC2 (+)] (Sugai et al., 2008; Lee et al., 2009b; Takano et al., 2009; Namikawa and Hanazaki, 2010). In the present study, we observed that all of the GI-type GHPs had Incomp I type mucin stains. Yao et al. (2002) analyzed 22 GHPs with epithelial neoplasms and demonstrated that the incomplete intestinal mucin phenotype was more frequently found in the dysplastic or cancerous GHPs. Moreover, the two major histological gastric carcinoma types, intestinal- and diffuse-type, appear to be associated with different genetic pathways (Wu et al., 1997; Jiao et al., 2004). Many studies have demonstrated that LOH is fairly common in intestinal-type gastric cancer but is rare in diffuse-type tumors (Wu et al., 1997; Jiao et al., 2004). These findings suggest that diffuse-type gastric cancer is distinct from intestinal gastric carcinoma and that it has its own initiation and progression mechanisms. Taken together, our results suggest that GI-type GHPs may represent an intermediate stage in the process of malignant transformation that leads mainly to intestinal-type gastric carcinoma. This hypothesis needs to be clarified in future studies involving a larger number of cases.

In conclusion, the mucin phenotype may be a useful marker for the malignant potential of GHPs, and GI-type GHPs should be considered to have malignant potential. Patients older than 50 years with GI-type GHPs should be considered at risk for gastric cancer.

Acknowledgements. We wish to thank Jin LIU for her excellent technical assistance.

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Accepted August 23, 2013