We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 5

Fetal-type Glycogen Phosphorylase (FGP)Expression in Intestinal Metaplasia as a High Risk Factor of the Development of Gastric Carcinoma

Masafumi Kuramoto, Shinya Shimada, Satoshi Ikeshima, Kenichiro Yamamoto, Toshiro Masuda, Tatsunori Miyata, Shinichi Yoshimatsu, Masayuki Urata and Hideo Baba

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/51793

1. Introduction

It has been reported that an enzyme-glycogen phosphorylase [EC 2.4.1.1] histochemical reaction is observed in differentiated hepatic or muscular tissues and in some proliferating tissues including fetus and carcinoma [1,2]. In the human stomach, a phosphorylase reaction appears in the undifferentiated gastric epithelium at the midpoint of fetal life, and is not detected in gastric epithelium after birth.

In our previous study we hitochemically demonstrated intense glycogen phosphorylase (GP) activity in gastric cancer cells, especially well-differentiated adenocarcinoma, and in the proliferative zone of some intestinal metaplasia (IM), despite phosphorelase being negative in normal gastric epithelium, even in its proliferative zone. Detailed histochemical observations of the enzyme activity were undertaken on the whole mucous membrane of surgically resected stomachs. A positive reaction was observed in all of the well-differentiated adenocarcinomas, whereas only a few poorly differentiated adenocarcinomas reacted positively. A positive reaction of the proliferative zone was observed in 69.5% of all metaplastic glands of the stomachs with well-differentiated adenocarcinoma, in 25.7% with poorly differentiated adenocarcinoma, and only rarely in glands from patients with peptic ulcer. Moreover, there was an apparent coincidence between the location of well-differentiated ad-

enocarcinoma and the distribution of IM with the proliferative zone showing a positive reaction for GP.

GP plays a central role in the mobilization of carbohydrate reserves in a wide variety of organs and tissues [3,4]. Mammalian GPs are found in three major isoforms, i. e., muscle, liver and brain that can be distinguished by functional and structural properties, as well as by the tissues in which they are predominantly expressed [4-6]. cDNAs encoding the three human GP isoforms have been cloned and sequenced, and the tissue and organism-specific expression patterns and chromosomal localization of GP genes has been clarified [4,7]. Chromosome mapping analyses have revealed that the genes encoding muscle, liver and brain GP are assigned to chromosomes 11, 14, and 20, respectively, suggesting that distinct *cis*-acting elements govern the differential expression of the phosphorylase isoforms in various tissues. The physiological role of muscle and liver GP is to provide fuel for the energy production required for muscle contraction and to ensure a constant supply of glucose for extrahepatic tissues, respectively. However, the physiological role of brain GP is poorly understood, although brain GP is generally thought to induce an emergency glucose supply during a stressful and/or ischemic period [4,6,8,9]. In addition, it has been proven that the major isoform of GP found in fetal tissue and tumor tissue is brain GP, and brain GP is identical to fetal-type GP (FGP) [8,9].

We developed an immunohistochemical method of detecting GP isoforms in human tissues by using specific antibodies raised against highly purified GP isoforms from rat brain, muscle and liver, and immunohistochemical staining of GP isoforms was undertaken to define the type of GP present in well-differentiated adenocarcinoma and in the proliferative zone of IM of the human stomach. Both the malignant cells of well-differentiated adenocarcinoma and the proliferative zone of some IM of the stomach were stained when the anti-FGP antibody was used, but not when the other two types were used. The results suggested that the newly appearing GP in gastric carcinoma was FGP, and it could be one example of fetal protein expression in cancer, like α-fetoprotain or carcinoembryonic antigen. Moreover, the proliferative zone of some IM having FGP (FGP-positive IM) might histogenetically relate to well-differentiated adenocarcinoma, i. e., FGP-positive IM could be regarded as a precursor of well-differentiated adenocarcinoma [10].

2. Novel subtyping of IM according to FGP expression

It is generally accepted that IM in the stomach increases the risk of gastric carcinoma [11-15]. A paucity of gene rearrangements is common to IM and carcinoma, which makes it difficult to establish a direct carcinogenic link between them. IM has been classified into subtypes with the aim of clarification of gastric carcinogenesis according to different definitions of the subtypes from the viewpoints of morphologic, enzymatic, and mucin-secreting patterns [16-19]. In such a classification, the subtyping is complicated and subjective, resulting in the existence of many variants within it. And also, some studies suggested that IM was a good marker for high risk of gastric cancer but the subclassification of IM was not important

http://dx.doi.org/10.5772/51793

[13,20]. A better classification of IM related to carcinogenesis that resolved the discrepancy between these two opinions would contribute to studying the direct link between gastric cancer and IM and to follow-up of the high-risk group.

To establish a noble classification of IM from a carcinogenic viewpoint, we studied 136 specimens with gastric carcinoma and the adjacent IM, that were obtained from gastric cancer patients (intestinal type, 72 patients; diffuse type, 64 patients), using specific anti-FGP antibody, and assessed how FGP expression correlates with subtypes of IM, proliferating cell nuclear antigen-labeling index, and various oncogene products.

	Intestinal-Type Carcinoma (n = 72)		***IM (n =64)***	
	No. (%) BGP Positive	***No. (%) BGP Negative***	***No. (%) BGP Positive***	***No. (%) BGP Negative***
	58 (80)*	14 (19)	56 (88)*	8 (12)
p53 Positivity (%)	42 (72.4)	8 (57.1)	10 (17.9)	0 (0.0)
	Diffuse-Type Carcinoma (n = 64)		***IM (n = 24)***	
	No. (%) BGP Positive	***No. (%) BGP Negative***	***No. (%) BGP Positive***	***No. (%) BGP Negative***
	12 (19)*	52 (81)	10 (42)*	14 (58)
p53 Positivity (%)	2 (16.7)	10 (19.2)	0 (0.0)	0 (0.0)

BBGP = brain-type glycogen phosphorylase; IM = intestinal metaplasia.

*P < .001.

Table 1. Incidence of FGP and p53 positivity in gastric carcinoma and IM.

2.1. FGP expression in gastric carcinoma and IM

As shown in Table 1, 80% (58/72) of the intestinal type carcinoma expressed FGP, while 19% (12/64) of the diffuse type showed positive staining for FGP. The percentage of the immunohistochemical positivity for anti-FGP was significantly greater in intestinal type than in diffuse type carcinoma ($P < 0.001$). The IM adjacent to carcinoma was found in 88% (64/72) and 38% (24/64) of intestinal and diffuse type carcinoma cases, respectively. In the intestinal type, 88% (56/64) of the adjacent IM showed FGP positivity, on the other hand, 42% (10/24) of the adjacent IM showed anti-FGP antibody reactivity in the diffuse type. The expression of FGP in the IM adjacent to intestinal carcinoma was significantly higher than in the diffuse type ($P < 0.001$).

2.2. Relationship between complete and incomplete IM and expression of FGP in the generative cells

The expressions of FGP in the generative zone of IM were compared with the type of complete or incomplete IM. We selected 64 cases in which the adjacent mucosa to intestinal-type carcinoma was IM. The morphologic and mucin-histochemical examination revealed that 23 (36%) and 41 (64%) cases of the adjacent IM were complete and incomplete, respectively.

The incidence of incomplete-type IM adjacent to the intestinal type of cancer was significantly higher than that of complete-type IM. However, there was no significant relationship between the conventional subtyping of IM and the expressions of FGP. The FGP expressions in complete and incomplete IM adjacent to the carcinoma were high in both of them (19/23, 82.6%; 37/41, 90.6%; respectively).

2.3. Proliferating state and FGP-positive IM

The proliferative compartment measured by proliferating cell nuclear antigen (PCNA) staining in the FGP-negative IM tend to be confined to the lower layer. In the FGP-positive IM, however, the PCNA labeling was frequently expanded to the upper layer. The labeling index analysis revealed that the index of the FGP-positive IM was significantly higher than that of the FGP-negative IM, although there was not a significant difference between PCNA labeling index of FGP-positive carcinoma and that of FGP-negative carcinoma (Figure 1).

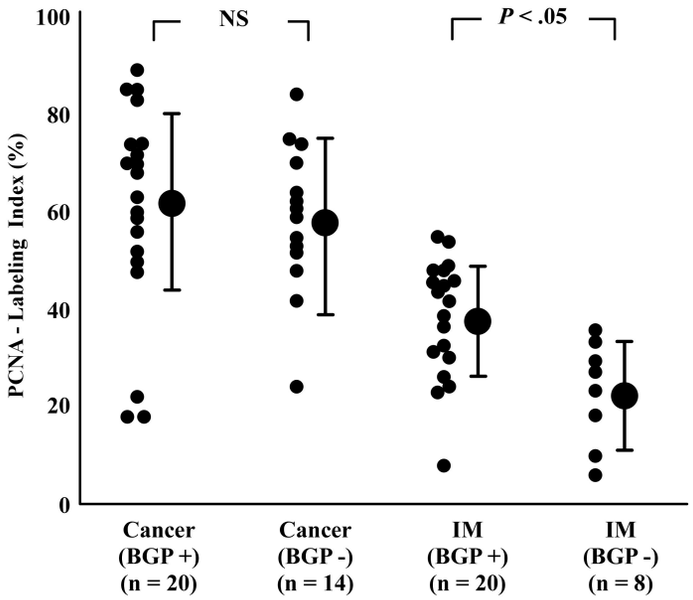

Figure 1. Distribution of PCNA labeling index in intestinal-type gastric cancer and adjacent IM with or without FGP expression. NS: not significant

2.4. Relevance of p53 expression with FGP-positive IM

Abnormal p53 accumulation was observed in 10 of 56 (17.9%) of the FGP-positive IM adjacent to intestinal carcinoma (Table 1). The staining of p53 was restricted to the FGP-positive IM mainly in their generative zone (Figure 2), and none of the staining was detected in the FGP-negative IM. In cancer foci, the overexpression of p53 was observed in 42 of 58 (72.4%), 8 of 14 (57.1%), 2 of 12 (16.7%), and 10 of 52 (19.2%) in the intestinal-type carcinomas with or without FGP or the diffuse-type with or without FGP, respectively. The percentage of p53 staining of intestinal-type carcinoma was significantly higher than that of the diffuse-type (P

http://dx.doi.org/10.5772/51793

< 0.001), and the intestinal-type carcinoma with FGP tend to be stained with p53 more frequently than that without FGP. The immunohistochemical staining of APC and c-K-ras was consistently negative in IM.

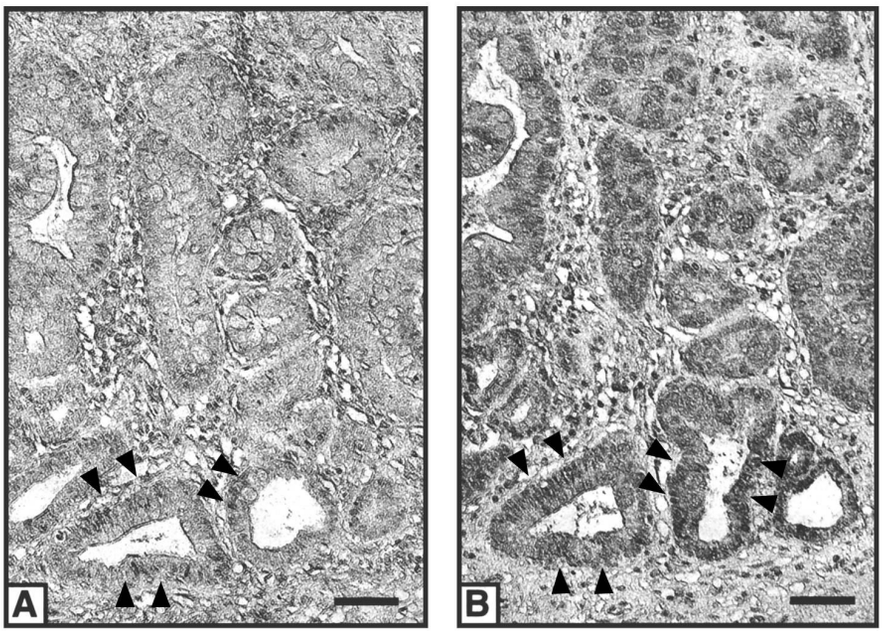

Figure 2. Overexpression of p53 protein in FGP-positive IM (arrows).A, p53 staining; B, FGP staining; Bar-100μm

In this study, FGP was expressed in 80% of the intestinal-type carcinoma and in 88% in the generative zone of IM adjacent to the cancer foci, whereas no positive staining was observed in the normal gastric mucosa, including its generative zone. The proportion of FGP positivity in cancer and IM was significantly greater in intestinal-type carcinoma than in the diffuse-type ($P < 0.001$). Thus, these results indicate an apparently close association between FGP-positive IM and intestinal-type gastric carcinoma. Interestingly, morphologic and mucin characterization revealed that there was no significant correlation between the subtypes of IM (i.e., complete or incomplete) and the expression of FGP in the generative cells of IM adjacent to intestinal-type carcinoma.

The characterization of FGP-positive IM was also conducted in this study, using the PCNA labeling index and the expression of oncogene products. PCNA staining revealed that there was an expansion of proliferative compartment in FGP-positive IM and it was significantly higher in a proliferating state than FGP-negative IM. A comparison of the PCNA labeling index of FGP-positive IM with that of FGP-negative IM indicated that the labeling index might predict more than 40% of the FGP positivity. Furthermore, some of the FGP-positive IMs were coexpressed accumulated p53 in the generative cells, although other oncogene products (APC and c-K-ras) that are common in the adenoma-carcinoma sequence in the colon [21] were detected in none of the generative cells of IM. PCNA and p53 have been considered to be crucial markers for the demonstration of cell proliferation in the cell cycle phase [22]. The timing of the genetic alteration of p53 has been investigated in the chain of

chronic gastritis, IM, dysplasia, and early carcinoma and reported to be an early event in stomach carcinogenesis [23-25]. Abnormal protein accumulation of p53, however, has not been well demonstrated in IM. In this study, we detected p53 accumulation in the generative cell zone of FGP-positive IM despite sporadic expression. These observations suggest that the generative cells of FGP-positive IM may deviate from the differentiation and be blocked from apoptotic cell death. Thus, this novel classification of IM based on the linkage between the generative cell zone of IM and gastric carcinoma using FGP expression may open new vistas in research of the carcinogenesis of gastric carcinoma.

3. Gastric and intestinal phenotypes of gastric carcinoma with reference to expression of FGP

Characterization of differentiated gastric carcinoma, i.e., gastric- and intestinal-phenotypic classification, has been advocated, mainly from the carcinogenic point of view, using analysis of the expression of gastric and/or intestinal mucin [26-29]. Carcinoma of the stomach has long been classified into differentiated type and undifferentiated types according to its histological morphologic characteristics [30]. Because the differentiated type is closely related to IM, and the tumor cells often have intestinal properties, the differentiated type is called intestinal type, while the undifferentiated type is called diffuse type [31]. Some differentiated-type carcinoma, however, are composed of cells resembling foveolar epithelium or pyloric gland cells, indicating that these carcinomas may arise from the proper gastric epithelium. In recent years, immunohistochemical staining with various mucins has been shown to discriminate mucins with characteristics of gastric and colonic epithelium, leading to a better understanding of the background of gastric tumorigenesis [32,33]. However, differentiated gastric carcinoma is classified into five subtypes, i.e., gastric type, gastric type-dominant mixed type, intestinal type-dominant mixed type, intestinal type, and null type according to the relative amount of gastric and intestinal mucins, and this classification is complicated and may be confusing [29].

In this section, we focused on the relationship between the expression of FGP and gastric and intestinal mucin in gastric carcinoma, and we propose that FGP expression is a simple marker for discriminating carcinomas with intestinal phenotype from those with gastric phenotype. Ninety-six tissues of gastric carcinoma surgically resected from the patients (differentiated type: 46, undifferentiated type: 50) were studied regarding correlation of FGP expression with intestinal and gastric phenotypes, determined histologically and immunohistochemically with the various anti-mucin antibodies (HGM, CD10, MUC2).

3.1. Proportions of gastric and intestinal phenotypes in gastric carcinoma

The FGP expression in gastric carcinoma was seen in 82.6% (38/46) of the differentiated type and 24.0% (12/50) of the undifferentiated type, and which corresponded well with the results of our previous study. Both differentiated and undifferentiated gastric carcinomas were classified into three subtypes, i.e., gastric, mixed and intestinal types, according to the

http://dx.doi.org/10.5772/51793

relative amount of gastric (HGM-positive) and intestinal (MUC2 and/or CD10-positive) mucins and the histological morphology. The proportions of gastric, mixed and intestinal types in differentiated gastric carcinoma were 13.0%, 47.8% and 39.2%, respectively, while hand, these proportions in undifferentiated gastric carcinoma were 56.0%, 32.0% and 12.0%, respectively [Table 2].

Histological type	Gastric type	Mixed type	Intestinal type	Total
Differentiated (%)	6 (13.0)	22 (47.8)	18 (39.2)	46 (100.0)
Undifferentiated (%)	28 (56.0)	16 (32.0)	6 (12.0)	50 (100.0)
Total (%)	34 (35.4)	38 (39.6)	24 (25.0)	96 (100.0)

Table 2. Incidence of gastric and intestinal phenotypes in gastric carcinoma.

3.2. Relationship between phenotype and FGP expression

Figure 3 shows combination graphs for the mucin phenotypes of gastric carcinoma and FGP expression in differentiated adenocarcinoma (A) and undifferentiated adenocarcinoma (B). In both differentiated and undifferentiated types, the phenotype of gastric and intestinal mucin expression corresponded very well with FGP expression, that is, almost all carcinomas with gastric type (92.3% and 97.1%, respectively) did not express FGP, whereas almost all with intestinal type (90.9% and 83.3%, respectively) expressed FGP. However, 97.3% of the mixed type of differentiated adenocarcinoma expressed FGP, while only 33.3% of the mixed type of undifferentiated carcinoma expressed FGP.

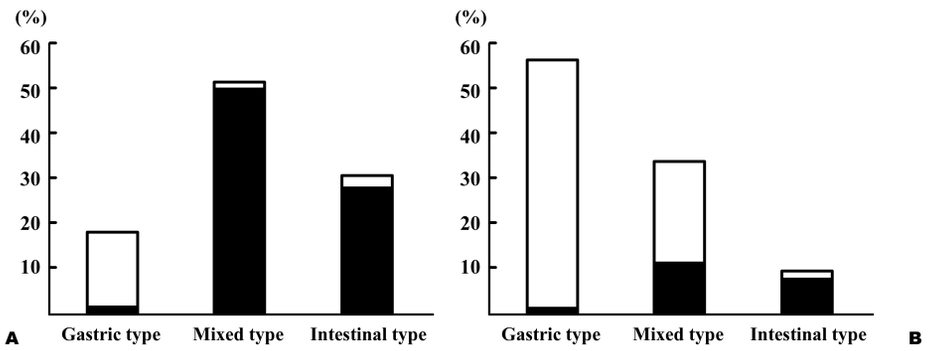

Figure 3. Combination graphs for mucin phenotype and FGP expression in A differentiated and B undifferentiated gastric carcinoma. *Open bars*, FGP-negative; *closed bars*, FGP-positive.

Recent progress in mucin histochemistry and immunohistochemistry has enabled us to differentiate the gastric and intestinal phenotypic properties of gastric carcinoma [C4, 7, 8]. However, in considerable numbers of gastric carcinomas that do not have typical and/or sufficient mucins (47.8% of the differentiated carcinomas in our study), it is difficult to decide the phenotype, and this leaves an equivocal group in this classification. Our study re-

vealed that almost all the phenotypes decided on by FGP expression corresponded with the results obtained with mucin immunohistochemistry and H&E staining, suggesting that FGP expression can discriminate the gastric and intestinal phenotypes in gastric carcinoma. We assume that this is not because FGP directly contributes to mucin carbohydrate expression, but probably because FGP-positive gastric carcinoma has intestinal-type lineage, leading to concordance with intestinal-type mucin expression findings. Accordingly, the mixed type carcinomas determined by mucin analysis were divided into two groups (gastric and intestinal types) according to FGP expression. Classification due to FGP expression can be achieved more easily, objectively, and simply than classification via the combined analysis of mucin immunohistochemistry.

Of note, this study also showed that the undifferentiated type of gastric cancer had gastric and intestinal phenotypes. Furthermore, in undifferentiated adenocarcinoma, the phenotype determined via mucin analysis corresponded with that determined via FGP expression. These results suggest that undifferentiated adenocarcinoma with FGP expression may arise from IM, while those without FGP expression arise from proper gastric epithelium, as has long been indicated [C5, 6].

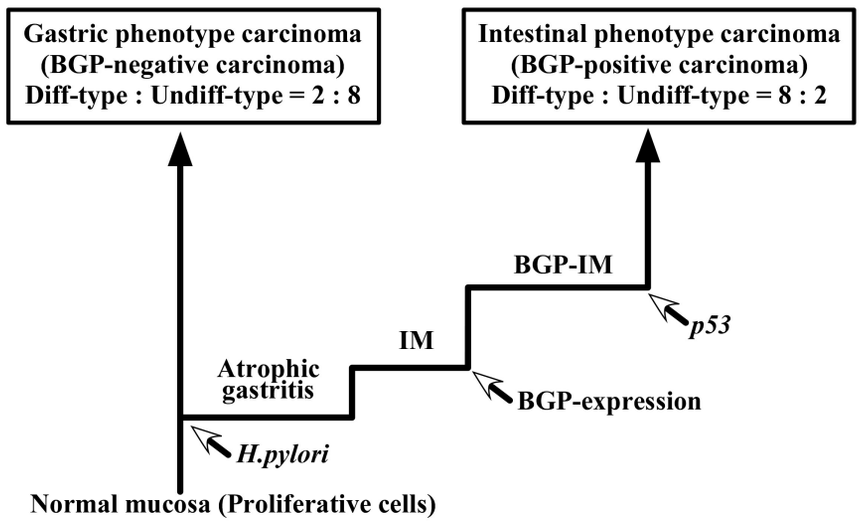

Figure 4. Relevance of FGP expression in the carcinogenesis of gastric and intestinal phenotypes of gastric carcinoma. *Diff-type*: Differentiated type, *Undiff-type*: Undifferentiated type, *IM*: intestinal metaplasia

A schema for our hypothesis derived from this study is shown in Figure 4. The ratios of differentiated and undifferentiated gastric cancers in FGP-positive and FGP-negative carcinomas were around 8:2 and 2:8, respectively. In a previous investigation, we found that FGP-positive proliferating cells in the IM appeared to be premalignant cells of intestinal-type carcinoma of the stomach. Therefore, it is suggested that approximately 80% and 20% of the differentiated and undifferentiated cancers, respectively, arise from FGP-positive proliferating cells of IM. On the other hand, approximately 20% and 80% of the differentiated and un-

http://dx.doi.org/10.5772/51793

differentiated cancers, respectively, arise from proper gastric mucosa, but candidates for the premalignant cells in proper gastric mucosa have not yet been suggested.

4. FGP expression in stomachs as a predictive risk factor for the synchronous and/or metachronous multiple gastric cancer

The recent advantage of endoscopic and laparoscopic local treatments has offered a better quality of life to patients with early gastric cancer involving no lymph node metastasis [34-38]. These treatments, however, incur increasing risks of missing the coexistence of accessory or microscopic carcinomas and/or developing new cancers in the remnant stomach [39-42]. The incidence of multiple primary gastric carcinomas has been reported to be from 5% to 10% in patients who had gastrectomy for gastric cancer [43-47]. The incidence is elevated with age and male sex, and with intestinal-type tumors; frequent occurrence in the lower third, and mucosal cancers, were significantly correlated with multiple early gastric cancer. However, these accessory lesions were missed preoperatively in approximately 30%-40% of the patients with multifocal early gastric cancers. Furthermore, considerable numbers of microscopic cancers could have been overlooked. Therefore, we should always remember that other lesions may also be present and/or grow when we are treating patients with gastric cancer by local treatment such as endoscopic treatment or laparoscopic wedge resection.

Local treatment for early gastric cancer is currently indicated mainly for intestinal-type carcinoma. If there were some indicators that predict the frequent coexistence of multiple gastric cancers and/or the metachronous growth of another gastric cancer of the intestinal-type, these would be very useful to identify the high-risk group and would contribute to the follow-up examinations after local treatment of gastric cancer patients. Our previous studies have demonstrated the significant role of the generative cells of FGP-positive IM as a premalignant lesion of intestinal-type gastric carcinoma.

Then, we designed the study to investigate the incidence of FGP-positive IM in gastric biopsy specimens and to establish FGP-positive IM as a predictor of the coexistence of accessory carcinoma and/or metachronous cancers before and after local treatment for early gastric cancer. Fifty-nine patients with intestinal-type early gastric cancer and endoscopic atrophic gastritis were analyzed. Of these patients, 14 had synchronous multiple gastric carcinomas, 25 had a single cancer, 20 had endoscopic atrophic gastritis without any localized lesions. During endoscopic examination, the lower two-thirds of the stomach was dyed with methylene blue and eight endoscopic biopsies were made from the stained mucosa in the anterior, posterior, greater and lesser curvature wall of the antrum and lower body of the stomach, respectively. Clinicopathological features of the patients showed that the patients with multiple early gastric carcinomas were significantly older than those with single early gastric carcinoma. However, there was no significant difference with the other parameters among the three groups.

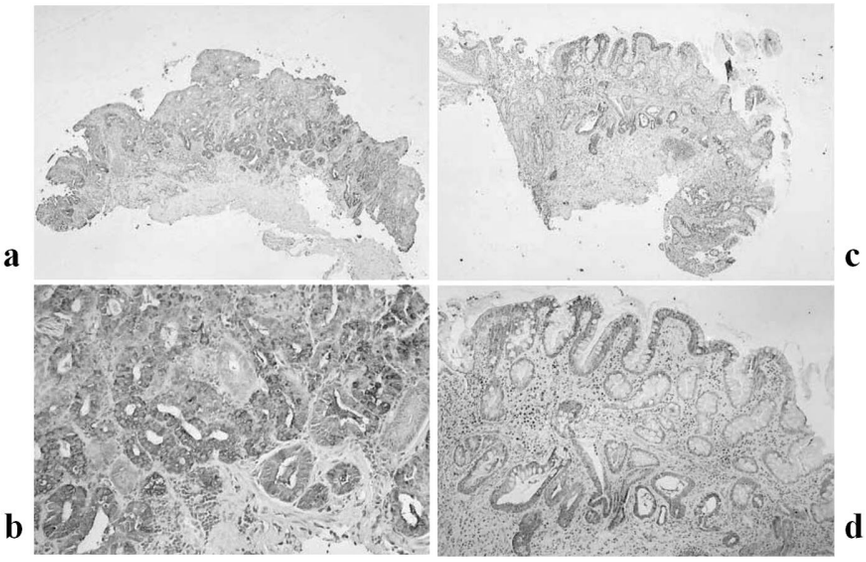

Figure 5. Immunohistochemical staining of biopsy specimens with anti-FGP antibody.**a** FGP-positive carcinoma, ×60; **b** high-power view, ×260; **c** FGP-positive IM, ×60; **d** high-power view, ×260

4.1. FGP expression in endoscopic biopsy specimens of gastric carcinoma and IM

Strongly positive reactivity was observed in the cytoplasm of cancer cells. In 93.3% (28/30) of the multiple carcinomas and 80.0% (20/25) of the single carcinomas, the biopsy specimens showed positive staining for FGP. The percentage of immunohistochemical positivity for anti-FGP antibody in the intestinal-type carcinoma corresponded well with previous our reports. The IM glands had structural deformity to a slight degree, but no cellular atypia. The generative cell zone of IM showed positive reactivity. Strong reactivity, similar to that in the cancer cells, was observed in the cytoplasm of the generative cells of IM (Figure 5).

4.2. Incidence of FGP-positive IM in stomachs with multiple carcinoma, single carcinoma and atrophic gastritis

Incidence of FGP-positive IM is shown in Figure 6. The distribution of the plots showing FGP-positive IM in the stomach was extremely characteristic in each group. The distribution was almost symmetrical in the multiple carcinoma and the atrophic gastritis groups. Although almost all stomachs with atrophic gastritis had no FGP-positive IM in any biopsy specimens, all the stomachs with multiple carcinomas had FGP-positive IM in each of the biopsy specimens. Furthermore, all the carcinomas in the multiple carcinoma group had high percentages of FGP-positive IM appearance, except for two in which FGP was negative in the cancer foci. On the other hand, a bipolarized distribution of the plots was observed in the single-carcinoma group; that is, about a quarter of the group had FGP-positive IM at high percentages, but about half of the group did not have it at all. The incidences of FGP-positive IM in the stomachs with multiple carcinomas, single carcinoma and atrophic gastritis were 83.2% ± 22.8%, 36.5% ± 41.3% and 7.1% ± 18.0%, respectively. The incidence of FGP-positive IM in the stomachs with multiple carcinomas was significantly higher than that in

http://dx.doi.org/10.5772/51793

those with a single carcinoma or those with atrophic gastritis. The incidence in stomachs with a single carcinoma was significantly higher than that in those with atrophic gastritis.

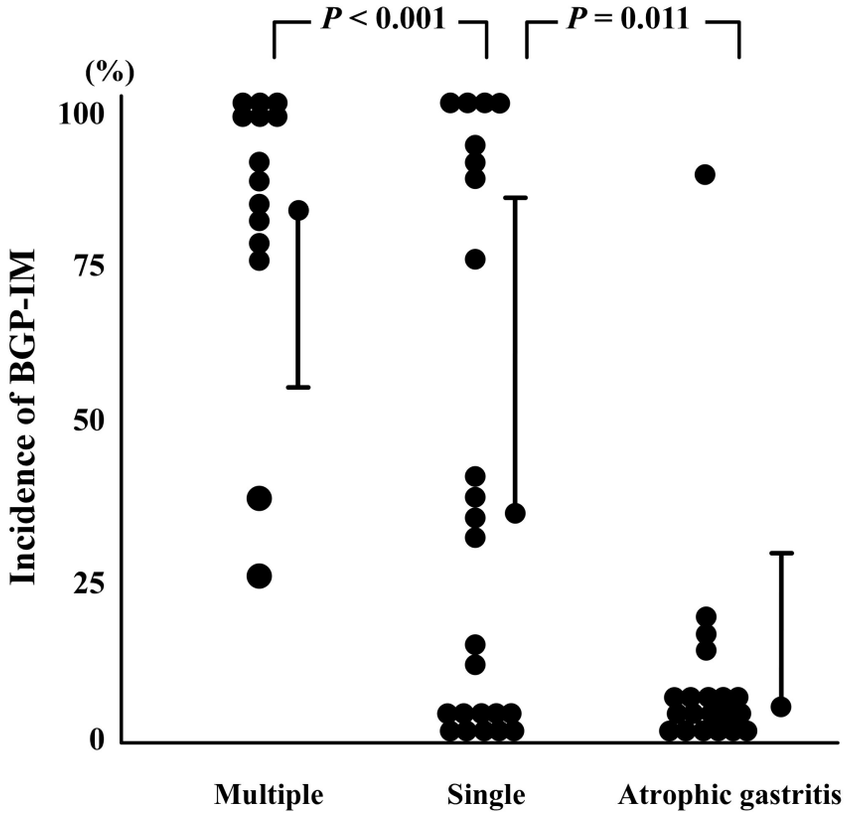

Figure 6. Distribution of incidence of FGP-positive IM in multiple carcinomas, single-carcinoma and atrophic gastritis groups.

4.3. Useful predictor for the development of new lesions after local treatment for early gastric cancer

One of the major problems with the local treatment of gastric cancer is that of the metachronous carcinomas in other parts of the stomach being different from the initial site of the carcinoma. A recent molecular biological study has suggested that high microsatellite instability in gastric tumors had a relationship with synchronous and/or metachronous gastric cancer compared with single carcinoma, whereas there was no difference in proliferative ability, carcinogenic pathway through p53 or K-ras, and various mismatch repair genes, although the mechanism was unclear [48]. However, the application of molecular genetics in the screening and surveillance of the patients with gastric cancer is still in its infancy. Arima et al. reported that metachronous recurrence was found in 6 of 76 endoscopically treated patients, and it was detected significantly more frequently in patients whose synchronous multiple lesions were found during the initial treatment; they stressed the importance of the

detection of gastric mucosal recurrence by frequent periodic endoscopic examinations during the follow-up period after the endoscopic treatment [41]. Early detection of the metachronous cancer is beneficial for the subsequent treatment of the new lesion, for which minimally invasive therapy, including endoscopic treatment, can be used. The necessity for frequent endoscopic follow-up, however, affects the quality of life for the patients and increases the overall medical cost. Therefore, a reliable predictive indicator of patients with a high risk of metachronous recurrence is very important for determining the schedule of endoscopic follow-up after the initial endoscopic treatment. Because metachronous recurrence was detected significantly more frequently in patients with synchronous multiple lesions [35,36,41], a predictive indicator for metachronous recurrence would correspond with the indicator for synchronous multiple gastric carcinoma.

Wittekind et al. analyzed 61 patients with synchronous gastric carcinoma from among 1664 patients, and suggested that multiple primary tumors arose from precancerous conditions leading to similar genetic alterations [47]. It is generally accepted that IM in the stomach increases the risk of gastric cancer [11-14]. However, it has been suggested that only 0.1-0.2% of IM is related to the carcinogenesis of intestinal-type gastric cancer worldwide [49]. Therefore, the IM significantly correlated with carcinogenesis of intestinal-type cancer should be selected for use as an appropriate marker. Our previous consecutive studies revealed that the proportion of FGP positivity in both cancer and IM was significantly greater in the intestinal-type carcinoma than in the diffuse-type; also, we found that FGP-positive IM had a much stronger correlation with gastric carcinoma than the conventional typing of IM, and FGP-positive IM was significantly higher in proliferating state than in those samples without FGP, and p53 mutation occurred only in FGP-positive IM, suggesting that FGP-positive IM is a precancerous condition for intestinal-type carcinoma [10,50-53]. Thus, our findings indicate that FGP-positive IM is an excellent marker of the early stage of gastric carcinogenesis.

We also clearly demonstrated that the incidence of FGP-positive IM appearance was significantly more frequent in the stomachs with multiple gastric carcinomas than in those with single carcinoma or those with atrophic gastritis. The finding that some of the stomachs with a single carcinoma had a high incidence of FGP-positive IM may suggest the coexistence of microscopic intestinal-type carcinomas or the possibility of metachronous recurrence in the future. Assay of FGP in IM by immunohistochemistry in endoscopic biopsy specimens is an easy and reliable technique to assess FGP-positive IM status in the stomach, and thus could serve as a predictor of the high potential of a stomach in which synchronous gastric carcinoma coexists for generating metachronous gastric carcinoma. These results suggest that the analysis of FGP expression in IM in biopsy specimens will contribute to the pre- and postoperative assessment of multiple and metachronous gastric cancer.

Most gastric cancers of the intestinal type are known to occur on the distal side of the endoscopic atrophic border [54,55]. We agree with both the opinion that “the surgeon is required to resect the area including the F-line at the time of distal gastrectomy so as not to leave another cancer in the gastric remnant” [54] and the opinion that “the treatment of multiple gastric cancer does not require extended operative procedure, and endoscopic resection may be indicated if each lesion fits the criteria for treatment and careful follow-up is ensured” [56].

http://dx.doi.org/10.5772/51793

The important thing is to have a good predictor for metachronous recurrence after local treatment [41,48]. Our study demonstrated that FGP-positive IM was detected even in the stomachs with endoscopic atrophic gastritis without any malignant lesion, suggesting that FGP-positive IM was not a pathological entity which was associated with the change of a carcinogenic microenvironment in the gastric mucosa. Therefore, FGP can serve as a potential predictor for the risk-assessment of the development of multiple and/or metachronous carcinomas. It may be possible to follow-up new lesions by this method, and follow-up studies will give better information on whether FGP-positive IM positivity could be a good predictor for metachronous recurrence after local treatment.

5. Conclusion

We have proposed that the novel classification of IM based on the linkage between the generative cell zone of IM and gastric carcinoma using FGP expression. And also, the classification of gastric and intestinal phenotypes of gastric carcinoma is simpler and clearer when FGP expression is used than when mucin immunohistochemical analysis is used. It is suggested that FGP is a useful biomarker for the classification of intestinal and gastric types of carcinoma of the stomach, including classification from the carcinogenic point of view. And lastly we demonstrated the importance of FGP-positive IM as a predictor for the metachronous recurrence of gastric carcinoma, and we propose immunohistochemical staining of FGP in multiple endoscopic biopsy specimens as a predictive indicator of synchronous cancer and/or metachronous recurrence.

Author details

Masafumi Kuramoto[1*], Shinya Shimada[1], Satoshi Ikeshima[1], Kenichiro Yamamoto[1], Toshiro Masuda[1], Tatsunori Miyata[1], Shinichi Yoshimatsu[2], Masayuki Urata[2] and Hideo Baba[3]

*Address all correspondence to: kuramoto@yatsushiro-gh.jp

1 Department of Surgery, Yatsushiro Social Insurance General Hospital, Yatsushiro, Japan

2 Department of Gastroenterology and Hepatology, Yatsushiro Social Insurance General Hospital, Yatsushiro, Japan

3 Department of Gastroenterological Surgery, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan

References

[1] Takeuchi, T., & Kuriaki, H. (1955). Histochemical detection of phosphorylase in animal tissues. *The Journal of histochemistry and cytochemistry*, 3(3), 153-160.

[2] Takeuchi, T., Miyayama, H., & Iwamasa, T. (1978). Histochemical demonstration of total phosphorylase activity for diagnosis of carcinoma cells in human stomach and intestines. *Stain technology*, 53(5), 257-260.

[3] Cori, C. F., & Cori, G. T. (1936). Mechanism and formation of hexosemonophosphate in muscle and isolation of a new phosphate ester. *Proceedings of the Society for Experimental Biology and Medicine*, 34, 702-712.

[4] Newgard, C. B., Hwang, P. K., & Fletterick, R. J. (1989). The family of glycogen phosphorylases: structure and function. *Critical reviews in biochemistry and molecular biology*, 24(1), 69-99.

[5] Shimada, S., Maeno, M., Akagi, M., Hatayama, I., Sato, T., & Sato, K. (1986). Immunohistochemical detection of glycogen phosphorylase isoenzymes in rat and human tissues. *The Histochemical journal*, 18(6), 334-338.

[6] Newgard, C. B., Littman, D. R., Genderen, C. V., Smith, M., & Fletterick, R. J. (1988). Human brain glycogen phosphorylase. Cloning, sequence analysis, chromosomal mapping, tissue expression, and comparison with the human liver and muscle isozymes. *The Journal of biological chemistry*, 263(8), 3850-3857.

[7] Nakano, K., Hwang, P. K., & Fletterick, R. J. (1986). Complete cDNA sequence for rabbit muscle glycogen phosphorylase. *FEBS letters*, 204(2), 283-287.

[8] Sato, K., Morris, H. P., & Weinhouse, S. (1972). Phosphorylase: a new isozyme in rat hepatic tumors and fetal liver. *Science*, 178(4063), 879-881.

[9] Gelinas, R. P., Froman, B. E., Mc Elroy, F., Tait, R. C., & Gorin, F. A. (1989). Human brain glycogen phosphorylase: characterization of fetal cDNA and genomic sequences. *Molecular brain research*, 6(2-3), 177-85.

[10] Shimada, S., Maeno, M., Misumi, A., Takano, S., & Akagi, M. (1987). Antigen reversion of glycogen phosphorylase isoenzyme in carcinoma and proliferative zone of intestinal metaplasia of the human stomach. An immunohistochemical study. *Gastroenterology*, 93(1), 35-40.

[11] Morson, B. C. (1955). Intestinal metaplasia of the gastric mucosa. *British journal of cancer*, 9(3), 365-376.

[12] Ming, S. C., Goldman, H., & Freiman, D. G. (1967). Intestinal metaplasia and histogenesis of carcinoma in human stomach. Light and electron microscopic study. *Cancer*, 20(9), 1418-1429.

[13] Stemmermann, G. N. (1994). Intestinal metaplasia of the stomach. A status report. *Cancer*, 74(2), 556-564.

[14] Correa, P., & Shiao, Y. H. (1994). Phenotypic and genotypic events in gastric carcinogenesis. *Cancer research*, 54(7), 1941-1943.

[15] Hill, M. J. (1994). Mechanisms of gastric carcinogenesis. *European journal of cancer prevention*, 3(2), 25-9.

[16] Tosi, P., Filipe, M. I., Baak, J. P., Luzi, P., Santopietro, R., Miracco, C., Sforza, V., & Megha, T. (1990). Morphometric definition and grading of gastric intestinal metaplasia. *The Journal of pathology*, 161(3), 201-208.

[17] Filipe, M. I., Muñoz, N., Matko, I., Kato, I., Pompe-Kirn, V., Jutersek, A., Teuchmann, S., Benz, M., & Prijon, T. (1994). Intestinal metaplasia types and the risk of gastric cancer: a cohort study in Slovenia. *International journal of cancer*, 57(3), 324-329.

[18] Iida, F., Murata, F., & Nagata, T. (1978). Histochemical studies of mucosubstances in metaplastic epithelium of the stomach, with special reference to the development of intestinal metaplasia. *Histochemistry*, 56(3-4), 229-37.

[19] Matsukura, N., Suzuki, K., Kawachi, T., Aoyagi, M., Sugimura, T., Kitaoka, H., Numajiri, H., Shirota, A., Itabashi, M., & Hirota, T. (1980). Distribution of marker enzymes and mucin in intestinal metaplasia in human stomach and relation to complete and incomplete types of intestinal metaplasia to minute gastric carcinomas. *Journal of the National Cancer Institute*, 65(2), 231-240.

[20] Kato, Y., Kitagawa, T., Yanagisawa, A., Kubo, K., Utsude, T., Hiratsuka, H., Tamaki, M., & Sugano, H. (1992). Site-dependent development of complete and incomplete intestinal metaplasia types in the human stomach. *Japanese journal of cancer research*, 83(2), 178-183.

[21] Fearon, E. R., & Vogelstein, B. (1990). A genetic model for colorectal tumorigenesis. *Cell*, 61(5), 759-767.

[22] Brito, M. J., Filipe, M. I., & Morris, R. W. (1992). Cell proliferation study on gastric carcinoma and non-involved gastric mucosa using a bromodeoxyuridine (BrdU) labelling technique. *European journal of cancer prevention*, 1(6), 429-435.

[23] Ranzani, G. N., Luinetti, O., Padovan, L. S., Calistri, D., Renault, B., Burrel, M., Amadori, D., Fiocca, R., & Solcia, E. (1995). p53 gene mutations and protein nuclear accumulation are early events in intestinal type gastric cancer but late events in diffuse type. *Cancer epidemiology biomarkers & prevention*, 4(3), 223-231.

[24] Tahara, E. (1993). Molecular mechanism of stomach carcinogenesis. *Journal of cancer research and clinical oncology*, 119(5), 265-272.

[25] Shiao, Y. H., Rugge, M., Correa, P., Lehmann, H. P., & Scheer, W. D. (1994). p53 alteration in gastric precancerous lesions. *The American journal of pathology*, 144(3), 511-517.

[26] Tatematsu, M., Ichinose, M., Miki, K., Hasegawa, R., Kato, T., & Ito, N. (1990). Gastric and intestinal phenotypic expression of human stomach cancers as revealed by pep-

sinogen immunohistochemistry and mucin histochemistry. *Acta pathologica japonica*, 40(7), 494-504.

[27] Kushima, R., & Hattori, T. (1993). Histogenesis and characteristics of gastric-type adenocarcinomas in the stomach. *Journal of cancer research and clinical oncology*, 120(1-2), 103-11.

[28] Akashi, H., Hinoda, Y., Itoh, F., Adachi, M., Endo, T., & Imai, K. (1997). A novel gastric-cancer-associated mucin antigen defined by a monoclonal antibody A3D4. *International journal of cancer*, 73(6), 795-801.

[29] Egashira, Y., Shimoda, T., & Ikegami, M. (1999). Mucin histochemical analysis of minute gastric differentiated adenocarcinoma. *Pathology international*, 49(1), 55-61.

[30] Nakamura, K., Sugano, H., & Takagi, K. (1968). Carcinoma of the stomach in incipient phase: its histogenesis and histological appearances. *Gann*, 59(3), 251-258.

[31] Lauren, P. (1965). The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo- clinical classification. *Acta pathologica et microbiologica Scandinavica*, 64, 31-49.

[32] Bara, J., Gautier, R., Mouradian, P., Decaens, C., & Daher, N. (1991). Oncofetal mucin M1 epitope family: characterization and expression during colonic carcinogenesis. *International journal of cancer*, 47(2), 304-310.

[33] Tytgat, K. M., Büller, H. A., Opdam, F. J., Kim, Y. S., Einerhand, A. W., & Dekker, J. (1994). Biosynthesis of human colonic mucin: Muc2 is the prominent secretory mucin. *Gastroenterology*, 107(5), 1352-63.

[34] Tada, M., Murakami, A., Karita, M., Yanai, H., & Okita, K. (1993). Endoscopic resection of early gastric cancer. *Endoscopy*, 25(7), 445-450.

[35] Takekoshi, T., Baba, Y., Ota, H., Kato, Y., Yanagisawa, A., Takagi, K., & Noguchi, Y. (1994). Endoscopic resection of early gastric carcinoma: results of a retrospective analysis of 308 cases. *Endoscopy*, 26(4), 352-358.

[36] Kojima, T., Parra-Blanco, A., Takahashi, H., & Fujita, R. (1998). Outcome of endoscopic mucosal resection for early gastric cancer: review of the Japanese literature. *Gastrointestinal endoscopy*, 48(5), 550-554.

[37] Ohgami, M., Kumai, K., Otani, Y., Wakabayashi, G., Kubota, T., & Kitajima, M. (1994). Laparoscopic wedge resection of the stomach for early gastric cancer using a lesion-lifting method. *Digestive surgery*, 11, 64-67.

[38] Roukos, D. H. (1999). Current advances and changes in treatment strategy may improve survival and quality of life in patients with potentially curable gastric cancer. *Annals of surgical oncology*, 6(1), 46-56.

[39] Janssen, C. W., Jr, Lie, R. T., Maartmann-Moe, H., & Matre, R. (1990). Who gets a second primary cancer after gastric cancer surgery? *European journal of surgical oncology*, 16(3), 195-199.

[40] Seto, Y., Nagawa, H., & Muto, T. (1996). Treatment of multiple early gastric cancer. *Japanese journal of clinical oncology*, 26(3), 134-138.

[41] Arima, N., Adachi, K., Katsube, T., Amano, K., Ishihara, S., Watanabe, M., & Kinoshita, Y. (1999). Predictive factors for metachronous recurrence of early gastric cancer after endoscopic treatment. *Journal of clinical gastroenterology*, 29(1), 44-47.

[42] El -Zimaity, H. M., & Ota, H. (1999). Endoscopic resection for early gastric cancer: possibilities and limitations. *Journal of clinical gastroenterology*, 29(1), 5-6.

[43] Honmyo, U., Misumi, A., Murakami, A., Haga, Y., & Akagi, M. (1989). Clinicopathological analysis of synchronous multiple gastric carcinoma. *European journal of surgical oncology*, 15(4), 316-321.

[44] Kosaka, T., Miwa, K., Yonemura, Y., Urade, M., Ishida, T., Takegawa, S., Kamata, T., Ooyama, S., Maeda, K., Sugiyama, K., et al. (1990). A clinicopathologic study on multiple gastric cancers with special reference to distal gastrectomy. *Cancer*, 65(11), 2602-2605.

[45] Brandt, D., Muramatsu, Y., Ushio, K., Mizuguchi, Y., Itabashi, M., Yoshida, S., Moriyama, N., Nawano, S., Ishikawa, T., Yamada, T., et al. (1989). Synchronous early gastric cancer. *Radiology*, 173(3), 649-652.

[46] Kodera, Y., Yamamura, Y., Torii, A., Uesaka, K., Hirai, T., Yasui, K., Morimoto, T., Kato, T., & Kito, T. (1995). Incidence, diagnosis and significance of multiple gastric cancer. *The British journal of surgery*, 82(11), 1540-1543.

[47] Wittekind, C., Klimpfinger, M., Hermanek, P., & Tannapfel, A. (1997). Multiple simultaneous gastric carcinomas. *British journal of cancer*, 76(12), 1604-1609.

[48] Kawamura, A., Adachi, K., Ishihara, S., Katsube, T., Takashima, T., Yuki, M., Amano, K., Fukuda, R., Yamashita, Y., & Kinoshita, Y. (2001). Correlation between microsatellite instability and metachronous disease recurrence after endoscopic mucosal resection in patients with early stage gastric carcinoma. *Cancer*, 91(2), 339-345.

[49] Kubo, T. (1981). Etiology of intestinal metaplasia. *In: Takemoto T, Kawai K, Ida K, Suzuku S (eds). Intestinal metaplasia of the human stomach (in Japanese). Tokyo: Igaku-Tosho-Shuppan*, 27-31.

[50] Shimada, S., Maeno, M., Misumi, A., & Akagi, M. (1984). Histochemical study of phosphorylase in proliferating cells of intestinal metaplasia and carcinoma of the human stomach. *Scandinavian journal of gastroenterology*, 19(7), 965-970.

[51] Shimada, S., Honmyo, U., Yagi, Y., Ikeda, T., Ogawa, M., & Yokota, T. (1992). Expression of glycogen phosphorylase activity in minute gastric carcinoma. *The American journal of gastroenterology*, 87(9), 1230-1231.

[52] Matsuzaki, H., Shimada, S., Uno, K., Tsuruta, J., & Ogawa, M. (1998). Novel subtyping of intestinal metaplasia in the human stomach: brain-type glycogen phosphory-

lase expression in the proliferative zone and its relationship with carcinogenesis. *American journal of clinical pathology*, 109(2), 181-189.

[53] Shimada, S., Tashima, S., Yamaguchi, K., Matsuzaki, H., & Ogawa, M. (1999). Carcinogenesis of intestinal-type gastric cancer and colorectal cancer is commonly accompanied by expression of brain (fetal)-type glycogen phosphorylase. *Journal of experimental & clinical cancer research*, 18(1), 111-118.

[54] Kimura, K., & Takemoto, T. (1969). An endoscopic recognition of the atrophic border and its significance in chronic gastritis. *Endoscopy*, 1, 87-97.

[55] Yoshimura, T., Shimoyama, T., Fukuda, S., Tanaka, M., Axon, A. T., & Munakata, A. (1999). Most gastric cancer occurs on the distal side of the endoscopic atrophic border. *Scandinavian journal of gastroenterology*, 34(11), 1077-1081.

[56] Takeshita, K., Tani, M., Honda, T., Saeki, I., Kando, F., Saito, N., & Endo, M. (1997). Treatment of primary multiple early gastric cancer: from the viewpoint of clinicopathologic features. *World journal of surgery*, 21(8), 832-836.