



## Original article

# Phenotypic shift in human differentiated gastric cancers from gastric to intestinal epithelial cell type during disease progression

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### Abstract:

**Background.** The phenotypic expression of tumor cells is widely thought to resemble that of the tissue of origin. In the present study, to assess phenotypic changes that occur with disease progression, we investigated human differentiated gastric cancers at different depths of invasion for component cancer cell types.

**Methods.** Using a combined mucin histochemical and immunohistochemical approach, we classified surgical specimens of 301 differentiated gastric cancers into three types: gastric epithelial cell (G) type, intestinal epithelial cell (I) type and mixed gastric and intestinal (GI) type, according to the phenotypic differentiation of the component cancer cells. The relation between the phenotypic type of cancer and their depth of invasion was evaluated.

**Results.** The proportion of G type cancers was 41.4% in early (tumor invasion of mucosa or submucosa) cases, decreasing to 22.2% in advanced (tumor invasion of muscularis propria or deeper) cases, whereas the proportion of I type cancers increased with progressive disease from 23.5% to 31.1% ( $P < 0.01$ ). Cancers invading the subserosa or deeper included more I type cases and fewer G type than cancers limited to the mucosa ( $P < 0.01$ ). In most cases of each phenotypic type, intestinal metaplasia was recognized in the surrounding background mucosa, but no clear relation was shown between the phenotype of cancers and the degree of intestinal metaplasia in the background mucosa, suggesting that intestinal metaplasia is not always a preneoplastic lesion.

**Conclusions.** A phenotypic shift from G to I type expression was observed with the progression of human differentiated gastric cancers. Intestinalization may occur independently in cancerous and noncancerous gastric mucosa.

**Key words:** human differentiated gastric cancer, differentiation phenotype, phenotypic change, intestinalization, preneoplastic lesion

### Introduction

The phenotypic expression of tumor cells is widely thought to resemble that of the tissue of origin. Accordingly, examination of the phenotypic expression of gastric cancers should reveal their histogenesis. Developments in mucin histochemistry and immunohistochemistry have made it possible to readily determine the differentiation phenotype of individual gastric non-cancerous and cancerous cells. Using this approach, it is possible to classify human gastric cancer cells into four types of gastric or intestinal epithelial cell characteristics [1,2].

Histologically, human gastric carcinomas have been classified into two main groups, namely, the “intestinal” and “diffuse” types of Lauren [3]. The former corresponds to the “differentiated” type and the latter to the “undifferentiated” type of Nakamura et al. [4] and Sugano et al. [5]. The frequency of intestinal metaplasia in the surrounding mucosa has been compared in the two types, and consequently intestinal metaplasia has been considered as a preneoplastic lesion for the differentiated (“intestinal” type) adenocarcinoma [3–5].

We have previously shown that experimentally induced adenocarcinomas in rat glandular stomach consisted mainly of gastric epithelial cell type cancer cells, and that the intestinal epithelial cell type cancer cells appeared later in the larger tumors, suggesting a phenotypic shift from gastric to intestinal type expression during the course of tumor progression [6–10]. We have also shown that this phenotypic shift occurred in accordance with increasing depth of invasion in human gastric signet ring cell carcinomas, which are widely recognized to originate from gastric mucosa proper [11].

In the present study, to clarify the histogenesis and phenotypic shift that occurs with the progression of human differentiated gastric carcinomas, we evaluated 301 surgical specimens for their component cells, using a

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combined mucin histochemical and immunohistochemical approach.

## Patients and methods

### Samples

We evaluated specimens from 301 patients with primary differentiated gastric cancer (tubular [tub] 1, tub 2, and papillary [pap], according to the Japanese Classification of Gastric Carcinoma [12]) resected surgically at Aichi Cancer Center Hospital between 1990 and 1993. Of the 301 cancer patients, 231 were men and 70 were women; they ranged in age from 38 to 84 years (mean,  $62.7 \pm 9.3$  years).

All specimens were fixed in 10% buffered formalin. Carcinomas with adjacent nonneoplastic mucosa were cut serially into 5-mm slices in parallel with the lesser curvature and embedded in paraffin. Tissue sections stained with hematoxylin and eosin were examined and those with the deepest invasive front were selected for mucin histochemical and immunohistochemical approach. The depth of invasion was judged according to the criteria of the Japanese Classification of Gastric Carcinoma [12].

### Mucin histochemistry and immunohistochemistry

For mucin histochemistry, we employed galactose oxidase-Schiff (GOS) [13] and paradoxical concanavalin A staining (PCS) [14] techniques. For immunohis-

tochemistry, we used SH-9 [15,16], anti-pepsinogen II (Pg II) [17], TKH-2 [18,19], 91.9 H [20–22], and anti-sucrase [23] antibodies. The characteristics of each marker and the donors of the antibodies are listed in Table 1.

GOS-reactive mucins have been demonstrated in surface mucous cells of the fundic and pyloric mucosa. By PCS, mucins in the alimentary tract can be divided into two main classes: class III mucins in mucous neck cells, pyloric gland cells, and Brunner's gland cells and class II mucins in surface mucous cells, goblet cells, and the surface coat of intestinal absorptive cells. In this study, we used PCS identifying class III mucin. GOS and PCS staining procedures were performed as described previously [13,14]. The avidin-biotin-peroxidase complex (ABC) immunohistochemical method [24] (Vectastain Elite ABC kit, Burlingame CA, USA) was employed for immunohistochemistry. SH-9 reacts with nonneoplastic human tissues such as endocervical and bronchial epithelia and, in the alimentary tract, is specific for surface mucous cells of the fundic and pyloric mucosa. Immunohistochemical localization of Pg II has been demonstrated in mucous neck cells and chief cells of the fundic glands and mucous cells of the pyloric and Brunner's glands. TKH-2, in the alimentary tract, reacts with goblet cell mucin in normal small intestine and intestinal metaplasia. 91.9H, which was raised against purified normal colonic sulfomucin, is frequently positive in goblet cells and columnar cells in intestinal metaplastic glands of the stomach and gastric cancer cells, although it is completely negative in normal gastric

**Table 1.** Mucin histochemical and immunohistochemical differentiation markers

Mucin histochemistry	
Galactose oxidase-Schiff (GOS)	Positive for surface mucous cell type mucin.
Paradoxical concanavalin A staining (PCS) (type III mucin staining)	Positive for type III mucin (mucous neck cell, pyloric gland cell and Brunner's gland cell type mucin).
Immunohistochemistry	
SH9	Monoclonal antibody raised against a 49-kD peptide fragment of the CA-125 bearing antigenic molecule secreted from a human ovarian serous cystadenocarcinoma cell strain. Positive for surface mucous cells of the fundic and pyloric mucosa (kindly donated by Dr. Shunsuke Imai, Department of Pathology, Nara Medical University, Nara, Japan).
Pg II	Polyclonal antibody raised against human pepsinogen II. Positive for mucous neck and chief cells of the fundic glands, pyloric gland cells and Brunner's gland cells (kindly donated by Dr. Kazumasa Miki, Faculty of Medicine, the University of Tokyo, Tokyo, Japan).
TKH-2	Monoclonal antibody which recognizes mucin-associated sialyl Tn antigen. Positive for goblet cell mucin in normal small intestine and intestinal metaplasia (kindly donated by Dr. J.L. Werther, Gastrointestinal Research Laboratory, Mount Sinai Medical Center, NY, USA).
91.9H	Monoclonal antibody raised against purified sulfomucin from normal colonic mucosa. Positive for normal colonic epithelial cells and frequently positive for goblet cells and columnar cells in intestinal metaplastic glands of the stomach and gastric cancer cells (kindly donated by Dr. Tatsuro Irimura, Faculty of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan).
Sucrase	Polyclonal antibody raised against human sucrase. Positive for striated cell border of mature intestinal absorptive cells (kindly donated by Dr. Kazuyuki Hirano, Department of Pharmaceutics, Gifu Pharmaceutical University, Gifu, Japan).

mucosa. Anti-sucrase antibody reacts with the luminal surface of mature intestinal absorptive cells.

GOS, PCS class III mucin, SH-9, and Pg II are markers of the gastric epithelial cell (G) phenotype, whereas TKH-2, 91.9H, and sucrase are markers of the intestinal epithelial cell (I) phenotype.

#### Phenotypic expression of gastric cancer cells

Using the staining mentioned above, we classified gastric cancer cells into four phenotypic types (Table 2).

*Surface mucous cell (Su) type.* Cancer cells of this type contained GOS and/or SH-9 reactive mucins.

*Pyloric gland cell (Py) type.* Cancer cells of this type contained class III mucin and/or showed Pg II reactivity.

*Intestinal absorptive cell (Ab) type.* Cancer cells of this type formed tubular or papillary structures with incomplete striated cell borders and occasionally showed sucrase and/or 91.9H reactivity.

*Goblet cell (Go) type.* Cancer cells of this type contained TKH-2 and/or 91.9H-positive mucin.

The Su and Py types we classified as gastric epithelial cell (G) type cancer cells and the Ab and Go types as intestinal (I) type cancer cells.

#### Classification of cancers

Gastric cancers in which more than 90% of the sectional area consisted of G type cancer cells were classified as G type cancers and those containing I type cancer cells in more than 90% of their sectional areas were classified as I type cancers. Intermediate cases (i.e., cancers consisted of G and I type cancer cells, neither of which reaching more than 90% dominance) were classified as gastric and intestinal mixed (GI) type cancers and specimens in which most component cancer cells showed no reactions for any of the staining procedures used in this study were treated as unclassified.

#### Evaluation of intestinal metaplasia

The specimens were divided into three grades according to the extent of intestinal metaplasia of surrounding background mucosa: (1) minimal (intestinal metaplastic glands occupying less than 10% of mucosae), (2) moderate (intestinal metaplastic glands occupying between 10% and 90% of mucosae), and (3) marked (intestinal metaplastic glands occupying more than 90% of mucosae). As the nomenclature for intestinal metaplasia, we used Inada's recent classification, which relates to the cellular differentiation of metaplastic glands [25]. In that classification, intestinal metaplastic glands are divided into two main categories according to the presence or absence of gastric epithelial cell phenotype expression: (i) gastric and intestinal mixed type (GI type) intestinal metaplasia, and (ii) solely intestinal type (I type) intestinal metaplasia. In his original classification, each type is further divided into subtypes according to the component cells.

#### Statistical analysis

Ridit analysis was applied to compare the proportions of each photo type of gastric cancer in cancers with different depths of invasion.  $P < 0.01$  was regarded as statistically significant.

## Results

#### Phenotypic expression of gastric cancer cells

Many cases showed the coexistence of G and I type cancer cells. Some cancer cells had the characteristics of more than one type e.g., the simultaneous secretion of both SH-9- and TKH-2-positive mucin (Su type and Go type, respectively) was occasionally seen. However, the methods used in this study allowed the lesions to be

**Table 2.** Classification of gastric cancer cell phenotype based on mucin histochemical and immunohistochemical findings

PCS	Differentiation criteria							Phenotypic expression	
	Pg II	GOS	SH-9	TKH-2	91.9H	Sucrase	Striated cell border		
Class III	(+)	(-)	(-)	(-)	(-)	(-)	(-)	Py type	G type
Class II	(-)	(+)	(+)	(-)	(-)	(-)	(-)	Su type	
Class II	(-)	(-)	(-)	(+)	(+/-)	(-)	(-)	Go type	I type
Class II	(-)	(-)	(-)	(-)	(+/-)	(+/-)	(+)	Ab type	

PCS, Paradoxical concanavalin A staining; Pg II, pepsinogen II; GOS, galactose oxidase-Sciff; Py type, pyloric gland cell type; Su type, surface mucous cell type; Go type, goblet cell type; Ab type, intestinal absorptive cell type; G type, gastric epithelial cell type; I type, intestinal epithelial cell type

classified into G, GI, and I types according to the proportion of each type of component cells. Overall, of the 301 cases examined, 98 were G type, 89 were GI type, 81 were I type, and 33 cases were unclassified. In invasive cancers, there was no phenotypic difference between cancer cells in the mucosal area and those at the deep invasive front, irrespective of the histological expansive or infiltrative mode of infiltration.

#### *Organoid differentiation of intramucosal cancer cells*

Intramucosal cancer cases showing organoid differentiation [26,27] of cancer cells were frequent. These mimicked pyloric gland mucosa (Su type cancer cells occupying the upper, and Py type cancer cells occupying the lower regions; Fig. 1), GI type intestinal metaplastic mucosa (Su type, Go type, and Ab type cancer cells occupying the upper and Py type cancer cells occupying the lower regions; Fig. 2), or I type intestinal metaplastic mucosa (Go type and Ab type cancer cells occupying the mucosa; Fig. 3).

#### *Phenotypic shift of gastric cancer cells with disease progression*

The frequency of each phenotype of cancer in relation to depth of invasion is shown in Fig. 4. In the mucosal cancers limited to mucosa (m), including muscularis mucosae (mm), 40.4% were G type and 22.1% were I type, whereas in cancers invading as deep as subserosa (ss) 18.9% were G type and 43.2% were I type, and in

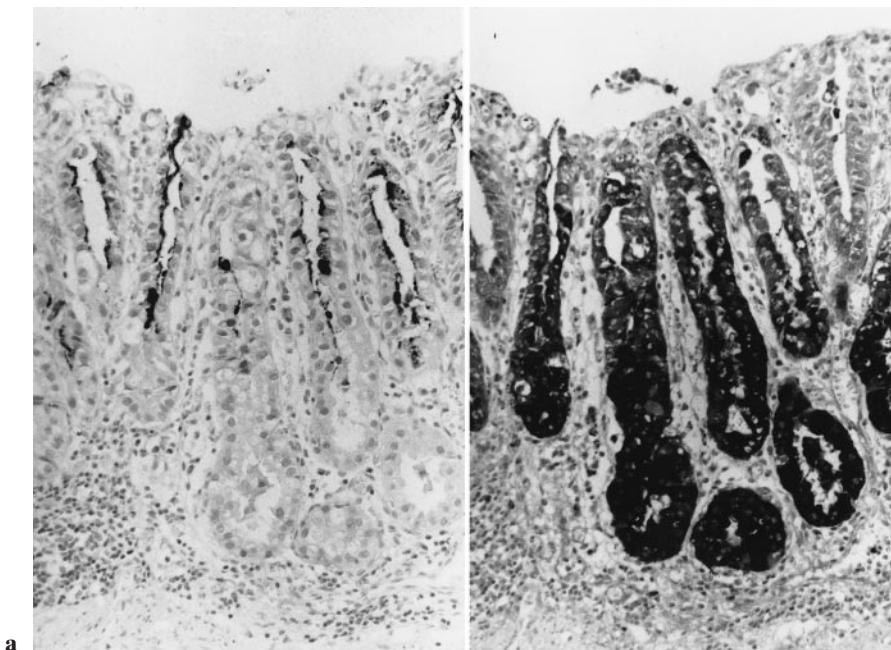
cancers invading the serosa and the peritoneal cavity (serosa-exposed, se) and invading in adjacent structures (serosa-infiltrating, si), 17.2% were G type and 26.6% were I type. The ss, se, and si cancers had significantly fewer G type and more I type cancers than the mucosal cancers ( $P < 0.01$ ). A comparison between early (tumor invasion of mucosa or submucosa) and advanced (tumor invasion of muscularis propria or deeper) cancers also showed a significant difference (G type, 41.0% vs 22.2%; I type, 23.5% vs 31.1%;  $P < 0.01$ ).

#### *Relation of cancer phenotype and surrounding mucosa*

In most cases of each phenotypic type, intestinal metaplasia was recognized in the surrounding background mucosa. Twenty-five of 98 G type cancers were surrounded by marked intestinal metaplastic mucosa and 15 by minimal metaplastic mucosa. Fifteen of 81 I type cancers were surrounded by minimal intestinal metaplasia and 16 by marked intestinal metaplasia. There was no clear relation between the phenotypic expression of the gastric cancers and the surrounding mucosa. In evaluation limited to mucosal cancers the finding was similar (Table 3).

## Discussion

In previous studies [1,2], we classified the differentiation of gastric cancer cells into four types according to staining with PCS, GOS, sialidase-GOS, and Pg I and II



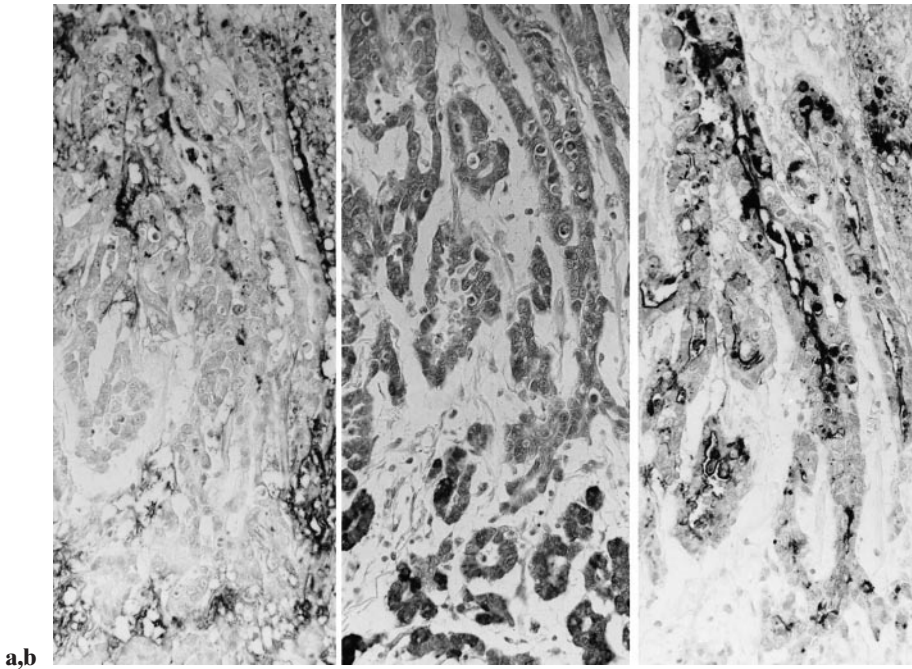
**Fig. 1a,b.** Gastric (G) type mucosal gastric cancer. **a** Stained with SH-9; **b** stained with anti-pepsinogen II (Pg-II). SH-9-positive surface mucous cell (Su) type cancer cells in the upper region and PgII-positive pyloric gland cell (Py) type cancer cells in the lower region, showing organoid differentiation mimicking pyloric gland mucosa (original magnification,  $\times 250$ )



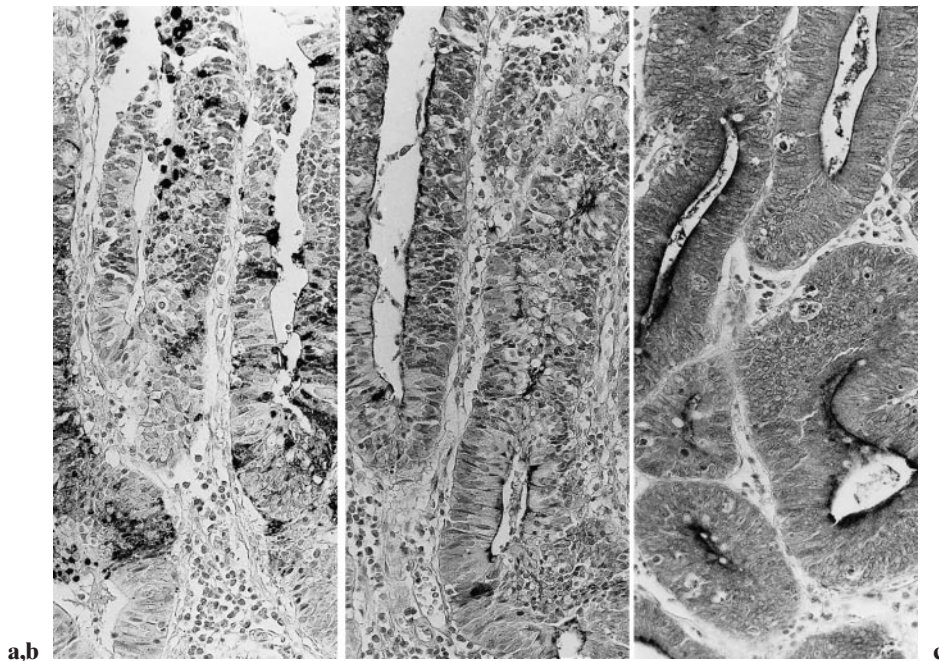
antibodies: Su and Py types (G type) and Ab and Go types (I type). In the present study, we added four new markers (SH-9, TKH-2, 91.9H, and anti-sucrase antibodies) to determine the differentiation of class III mucin-negative cells more precisely. In the normal adult human alimentary tract, sialomucin is dominant in the small intestine and sulfomucin in the large intestine. However, this distribution is not universal in other spe-

cies or in the developing human fetus [28–31], so in this study of cancer tissues, we did not regard TKH-2 or 91.9H as specific markers of the small or large intestinal phenotype, but used them as general markers of intestinal phenotype.

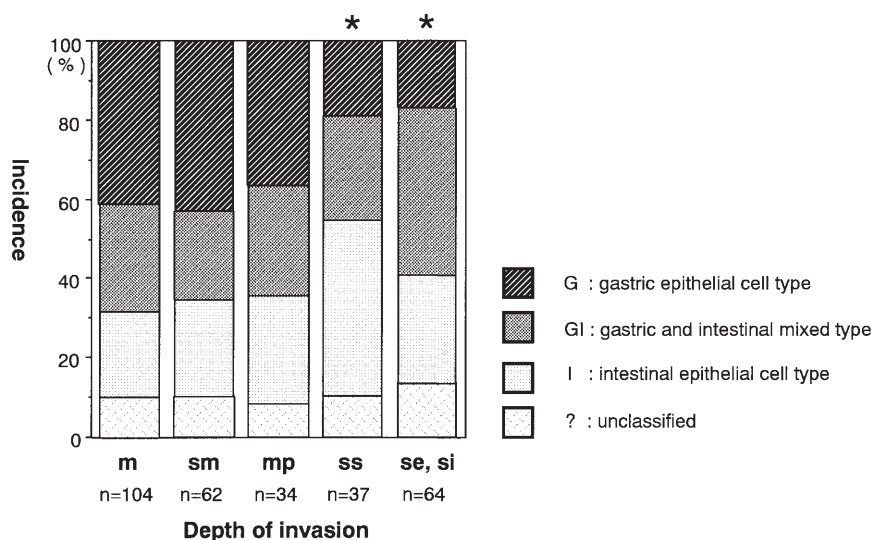
Lauren [3] classified gastric carcinomas as “intestinal” and “diffuse” types. Nakamura et al. [4] and Sugano et al. [5] classified gastric cancers as “differenti-



**Fig. 2a–c.** Mixed gastric and intestinal (GI) type mucosal gastric cancer. **a** Stained with SH-9; **b** stained with PgII; **c** stained with TKH-2. SH-9-positive Su type cancer cells and TKH-2 positive goblet cell (Go) type cancer cells were mixed in the upper region, and PgII-positive Py type cancer cells were recognized in the lower region, showing organoid differentiation mimicking GI type intestinal metaplasia (original magnification,  $\times 200$ )



**Fig. 3a–c.** Intestinal (I) type mucosal gastric cancer. **a** Stained with TKH-2; **b** stained with 91.9H, **c** stained with sucrase. Note intestinal absorptive cell (Ab) type cancer cells with sucrase expression and 91.9H-positive striated cell borders and scattered Go type cancer cells with TKH-2- and/or 91.9H-positive mucin showing I type differentiation (original magnification,  $\times 300$ )



**Fig. 4.** Depth of invasion and differentiation phenotype in differentiated gastric cancers. \* $P < 0.01$  vs m. *m*, mucosa; *sm*, submucosa; *mp*, muscularis propria; *ss*, subserosa; *se, si*, serosa-exposed, serosa-infiltrating

**Table 3.** Relation between phenotypic expression of differentiated gastric cancers and properties of surrounding mucosa

IM	Age (years)	No. of cases	G	GI	I	?
Mucosal cancer cases						
Minimal	62.9 ± 14.8	11	5	5	1	0
Moderate	60.7 ± 9.6	64	27	19	14	4
Marked	61.0 ± 8.7	29	10	5	8	6
Total	61.0 ± 10.0	104	42	29	23	10
All cases						
Minimal	61.8 ± 11.1	56	15	21	15	5
Moderate	63.0 ± 9.1	185	58	58	50	19
Marked	62.6 ± 8.3	60	25	10	16	9
Total	62.7 ± 9.3	301	98	89	81	33

Mucosal cancer, Invasion limited to mucosa, including muscularis mucosae

IM, Intestinal metaplasia; G, gastric epithelial cell type; GI, gastric and intestinal mixed type; I, intestinal epithelial cell type; ?, unclassified; minimal, <10% intestinal metaplasia; moderate, 10%–90% intestinal metaplasia; marked, >90% intestinal metaplasia

ated” and “undifferentiated” types (corresponding to Lauren’s “intestinal” and “diffuse” types respectively). With regard to the histogenesis of gastric cancer, the frequency of intestinal metaplasia in the surrounding mucosa was compared in the two types, and it was suggested that “intestinal” (“differentiated”) type carcinomas arose in the intestinalized mucosa and “diffuse” (“undifferentiated”) type carcinomas arose in the gastric mucosa proper [3–5]. However, contrary to this hypothesis, we found a high incidence of G type differentiated mucosal cancers and a phenotypic shift from G to I type expression in accordance with increasing depth of invasion. Yamachika et al. [32] also found a phenotypic shift from G to I type expression in their series of signet-ring cell carcinomas of the stomach, although

they found no I type mucosal cancer cases. The tendency for a phenotypic shift was commonly recognized with increasing depth of invasion with both histological types.

With mucosal cancers, we often found organoid differentiation mimicking pyloric gland mucosa, GI type intestinal metaplastic mucosa, and I type intestinal metaplastic mucosa. This suggests that similar courses of intestinalization may have been in progress in nonneoplastic gastric mucosa and in the mucosal cancers. Another interpretation of this phenomenon is that cancers mimicking intestinal metaplasia originated from intestinal metaplastic glands. Some differentiated gastric cancers may have had an intestinal phenotype from very early in their development, and we do not rule out

the possibility that the gastric cancers arose from already intestinalized glands showing intestinal metaplasia or gastric adenoma. GI type intestinal metaplasia with unstable cellular differentiation may give rise to early gastric cancers showing the GI phenotype. However, despite the presence of moderate to marked intestinal metaplasia in most of our cases (89.4% in mucosal cancer cases and 81.4% in all cases), 40.4% of differentiated mucosal cancers were G type and only 22.1% were I type. No clear relation between the phenotypic expression of cancer cells and their surrounding mucosa was shown, so it is difficult to say that intestinal metaplasia is a preneoplastic lesion for differentiated gastric cancers. Hattori [33] investigated for microcarcinomas by a serial-step section method with conventional staining and suggested that a starting point for adenocarcinomas and dysplasias was in the proliferative cell zone in the neck region. He concluded that intestinal metaplasia, dysplasia, and adenocarcinoma of the stomach arise coincidentally, with no precursor for any of them.

The apparent relation between the histological type of gastric cancer and intestinal metaplasia in the surrounding mucosa, together with the overall higher incidence of I type cancer cells in differentiated cancers, may reflect the phenotypic status of their background mucosa. Differentiated cancers do often develop in gastric mucosa with intestinal metaplasia. However, this does not mean that differentiated gastric cancers arise preferentially from intestinalized glands. This origin may be coincidental and it is possible that intestinal metaplasia and differentiated gastric cancers share a common background; in other words, intestinal metaplasia may be paraneoplastic, not preneoplastic. The background mucosa of differentiated gastric cancers may be prone to intestinalization. A common phenomenon observed among differentiated and undifferentiated gastric cancers and nonneoplastic gastric mucosa is the phenotypic shift to intestinalization, although the underlying mechanisms of this instability of cellular differentiation are yet to be elucidated.

In regard to the genetic background of intestinalization, changes in the expression of various genes determining cell structures and functions may be involved. Intestinalization differs from the nonsystematic deregulation of a single gene that is often seen during carcinogenesis; instead, it represents a kind of homeotic transformation [34,35]. Homeobox-containing genes (homeobox genes) play a key role in developmental processes. Recent evidence suggested the role of several homeobox genes in the malignant process [36–38]. Silberg et al. [36] showed that the homeobox gene *CDX1*, which codes for an intestine-specific transcription factor that may be involved in the regulation of proliferation and differentiation of intestinal epithelial

cells, was not expressed in normal stomach, but was expressed in intestinal metaplastic glands and sometimes in adenocarcinomas of the stomach. In the course of human ontogenesis, fetal stomach, which develops from the foregut, displays areas of “intestinal-type mucosa” with goblet cells and epithelial cells with striated borders in the antrum and cardia, as well as sulfomucin and sialomucin secretion [31,39]. Our findings for cancers may thus indicate a loss of ability to inhibit the primitive intestinal phenotype which was present in the fetal tissue but is normally inhibited in adult cells. A higher turnover rate of cells during development, regeneration, and carcinogenesis may lead to the appearance of cells that are less differentiated and possess the capacity to differentiate in both gastric and intestinal directions, perhaps with deregulation of homeobox gene expression.

We proposed earlier that the development of I type cells during experimentally induced rat gastric carcinogenesis occurred independently in nonneoplastic gastric mucosa and in gastric cancers [7]. We also showed that the intestinal phenotypic expression of cancer cells in rat stomach was stable and that the proportion of I type cancer cells was higher in larger tumors [8]. The findings of the present study are also consistent with the hypothesis that intestinalized cells may appear independently in normal gastric mucosa and in gastric cancers. Based on the idea of the monoclonal growth of tumors, it is assumed that individual cancers are derived from a single cell with intrinsic potential to differentiate into both G and I phenotypes, and the apparent phenotypic heterogeneity within cancers results from the phenotypic shift of cancer cells during progression. The observed phenotypic shift may be due to an intestinalization tendency in proliferating cancer cells or to differences in tumor biology (such as proliferative potential, apoptosis rates, and local aggressiveness) between G and I type cancer cells derived from the same original one cancer cell. The phenotypic shift from G to I type during the progression of gastric cancers may be of importance in the consideration of the biological behavior of gastric cancers in future studies.

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