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Intestinalization of gastric signet ring cell carcinomas with progression

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Abstract Recent developments in mucin histochemistry and immunohistochemistry have made reliable determination of the gastric and intestinal phenotypes of gastric carcinoma cells possible. Phenotypic expression changes from gastric epithelial cell type to intestinal epithelial cell type with the growth of gastric tumours in experimental animals. We studied cell differentiation in gastric signet ring cell carcinomas with progression in 203 surgically obtained specimens. The results showed that the proportion of gastric phenotype carcinomas, in which over 90% of the tissue consists of gastric epithelial cell type cells, decreases with the depth of invasion. The proportion of mixed phenotype carcinomas (between 10% and 90% of the tissue made up of gastric and/or intestinal epithelial cell type cells) increases. The intestinal phenotype (over 90% intestinal epithelial cell type carcinoma cells) was found in four carcinomas (about 2%) involving the serosa. No clear relationship was evident between phenotypic expression of carcinoma cells and the degree of intestinal metaplasia of the surrounding mucosa.

Progression of gastric signet ring cell carcinomas is associated with a phenotypic shift from gastric to intestinal type expression.

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Introduction

The phenotypic expression of tumour cells resembles that of the tissue of origin. Recent developments in mucin histochemistry {paradoxical concanavalin A (PCS) [17], galactose oxidase–Schiff (GOS) [18, 27] and sialidase GOS (S-GOS) [18, 27] staining} and immunohistochemistry {use of pepsinogen II (PgII) [6, 11, 13, 25, 26], SH-9 (for demonstrating CA125-bearing antigenic molecule fragments), [14, 34], and TKH-2 (for the sialosyl-Tn antigen) [15, 19]} have made reliable determination of the gastric and intestinal phenotypic expressions of gastric carcinoma cells possible.

Histologically, human gastric cacrinomas have been classified into two groups, the intestinal and diffuse types of Lauren [21] and the differentiated and undifferentiated types of Sugano et al. [28]. Cytologically, human gastric carcinoma cells can be classified into a gastric epithelial cell type (comprising surface mucous and pyloric gland cells) and an intestinal epithelial cell type (goblet and intestinal absorptive cell types) on the basis of their phenotypic expression [5, 7, 8, 15, 32–34]. Signet ring cell carcinomas consist of both gastric and intestinal epithelial type cells, which can be differentiated ultrastructurally into surface mucous, pyloric gland, goblet and microcyst types [2, 31].

We have previously shown that gastric adenocarcinomas induced experimentally in rats are mainly composed of tumour cells of the gastric epithelial cell type [29]. The phenotypic expression changes from gastric epithelial cell type to intestinal epithelial cell type with growth [30].

In this study, we investigated the variation in phenotypic expression of signet ring cell carcinomas with depth of invasion, from the mucosa through the submucosa and muscularis propria to the serosa. We chose to focus attention on this carcinoma because it produces

abundant mucin, which makes it particularly suitable for mucin histochemistry and immunohistochemistry techniques, and because it is widely recognized as originating from the gastric mucosa, regardless of the presence of metaplastic change [9, 22–24].

Materials and methods

In all, 203 surgical specimens of primary signet ring cell gastric carcinoma resected at Aichi Cancer Center Hospital and its affiliated hospitals during the period 1979–1992 were examined.

All specimens were fixed in 10% buffered formalin. Carcinomas were cut serially into 5-mm slices in parallel with the lesser curvature and then embedded in paraffin. Tissue sections were examined after staining with haematoxylin-eosin (HE) and those through the longest tumour diameters were selected for mucin histochemical and immunohistochemical investigation.

A signet ring cell gastric carcinoma was defined as a carcinoma with more than 50% of the carcinoma tissue composed of signet ring cells containing intracellular mucin.

For mucin histochemistry PCS, GOS and S-GOS staining techniques were applied. For immunohistochemistry SH-9, anti-Pg II and THK-2 antibodies were used. Reactive cells of each histochemical staining are shown in Table 1. Mucin identified by PCS was classified into four types. Reactive histological sites of class I are cytoplasmic membranes, those of class II are the surface epithelium of the stomach and goblet cells of the small intestine, those of class III are mucous neck cells, pyloric glands and Brunner's glands, and those of class IV are goblet cells of the large intestine [17]. In this study PCS identifying class III was used. GOS and S-GOS staining procedures were performed as described previously [18, 27]. The avidin-biotin-peroxidase complex (ABC) method [10] was employed to determine the localization of Pg II, SH-9-reactive mucin and TKH-2-reactive mucin in the gastric mucosa and carcinomas. Polyclonal anti-Pg II and TKH-2 monoclonal antibodies were prepared as described previously [12, 19]. The monoclonal antibody SH-9 was kindly donated by Dr. Shunsuke Imai (Department of Pathology, Nara Medical University) [14]. Affnity-purified biotin-labeled goat anti-rabbit or horse anti-mouse IgG and ABC were applied (Vectastain Elite ABC kit, Burlingame, Calif.). After deparaffinization and dehydration, sections were incubated with fresh 3% hydrogen peroxide in methanol and treated sequentially with normal goat or horse serum, rabbit anti-Pg II (1:4000) or mouse SH-9 monoclonal antibody (1:50) or TKH-2 (1:1000, respectively), biotin-labelled goat anti-rabbit or horse anti-mouse IgG, and ABC. The sites of peroxidase binding were visualized using diaminobenzidine. Sections were lightly counterstained with haematoxylin.

Carcinoma cells were classified into gastric epithelial cell (surface mucous and pyloric gland cell types) and intestinal epithelial cell (goblet and microcyst cells) types using their histochemical staining and HE staining features (Table 2) [5, 6, 15, 31–33]. Carcinomas with over 90% composed of gastric epithelial cells were classified as having the gastric phenotype, and those demonstrating over 90% intestinal epithelial cells, as having the intestinal phenotype. The remainder were assigned to the mixed phenotype.

The surrounding non-neoplastic gastric mucosa up to 5 mm from the margin of each carcinoma was examined, with division into four groups on the basis of location: cardiac gland region, fundic region, intermediate zone and pyloric gland region.

The Chi-square test for trend was applied for comparison of the proportions of each phenotype with different depths of invasion.

Table 1 Details for histochemically and immunohistochemically reactive cells in the stomach (*PCS* paradoxical concanavalin A, *GOS* galactose oxidase–Schiff, *S-GOS* sialidase galactose oxidase–Schiff, *Pg II* pepsinogen II)

Histochemistry	Reactive cells
PCS (class III)	Mucous neck cell Pyloric gland cell
GOS	Surface mucous cell
S-GOS	Surface mucous cell Goblet cell
Anti-PgII	Mucous neck cell Pyloric gland cell Chief cell
SH-9	Surface mucous cell
TKH-2	Goblet cell Parietal cell (weak)

Table 2 Classification of carcinoma cells by histochemical markers (*Gastric phenotype* gastric epithelial cell type, *Intestinal phenotype* intestinal epithelial cell type, *Sur. type* surface mucous cell type, *Pyl. type* pyloric gland cell type, *Gob. type* goblet cell type, *Mic. type* microcyst type)

Markers	Gastric phenotype		Intestinal phenotype	
	Sur. type	Pyl. type	Gob. type	Mic. type
PCS (class III)	–	+	–	–
GOS	+	–	–	–
S-GOS	+	–	+	–
Anti–PgII	–	+	–	–
SH-9	+	–	–	–
TKH-2	–	–	+	–
Microcyst	–	–	–	+

Results

With PCS, GOS and S-GOS staining and with anti-Pg II, SH-9 and TKH-2 immunohistochemistry, surface mucous cells contained GOS-, S-GOS- and/or SH-9-reactive mucin (Fig. 1a, b), and showed no class III mucin or Pg II staining. In contrast, mucous neck cells contained class III mucin and Pg II, and showed no GOS, S-GOS or SH-9 reactivities. Parietal cells showed no reactivity for any staining except TKH-2 immunohistochemistry. Chief cells demonstrated strong stainability for Pg II. Pyloric gland cells contained class III mucin and Pg II (Fig. 1c, d). Duodenal and intestinal metaplastic goblet cells contained S-GOS- and TKH-2-positive mucin, and no GOS- and SH-9-reactive mucin and no Pg II or class III positivity was demonstrable (Fig. 1e, f). Duodenal and intestinal metaplastic absorptive cells were covered with a surface coat characterized by slight S-GOS reactivity.

The phenotypes of signet ring cells were classified into surface mucous, pyloric gland, goblet and microcyst types. Carcinoma cells of surface mucous cell type contained GOS- and/or SH-9-reactive mucin but showed no class III mucin or Pg II reactivity. In contrast, carcinoma cells of pyloric gland cell type contained class III mucin and/or Pg II reactivity but showed no GOS or SH-9 reactivity. Carcinoma cells of goblet cell type contained S-

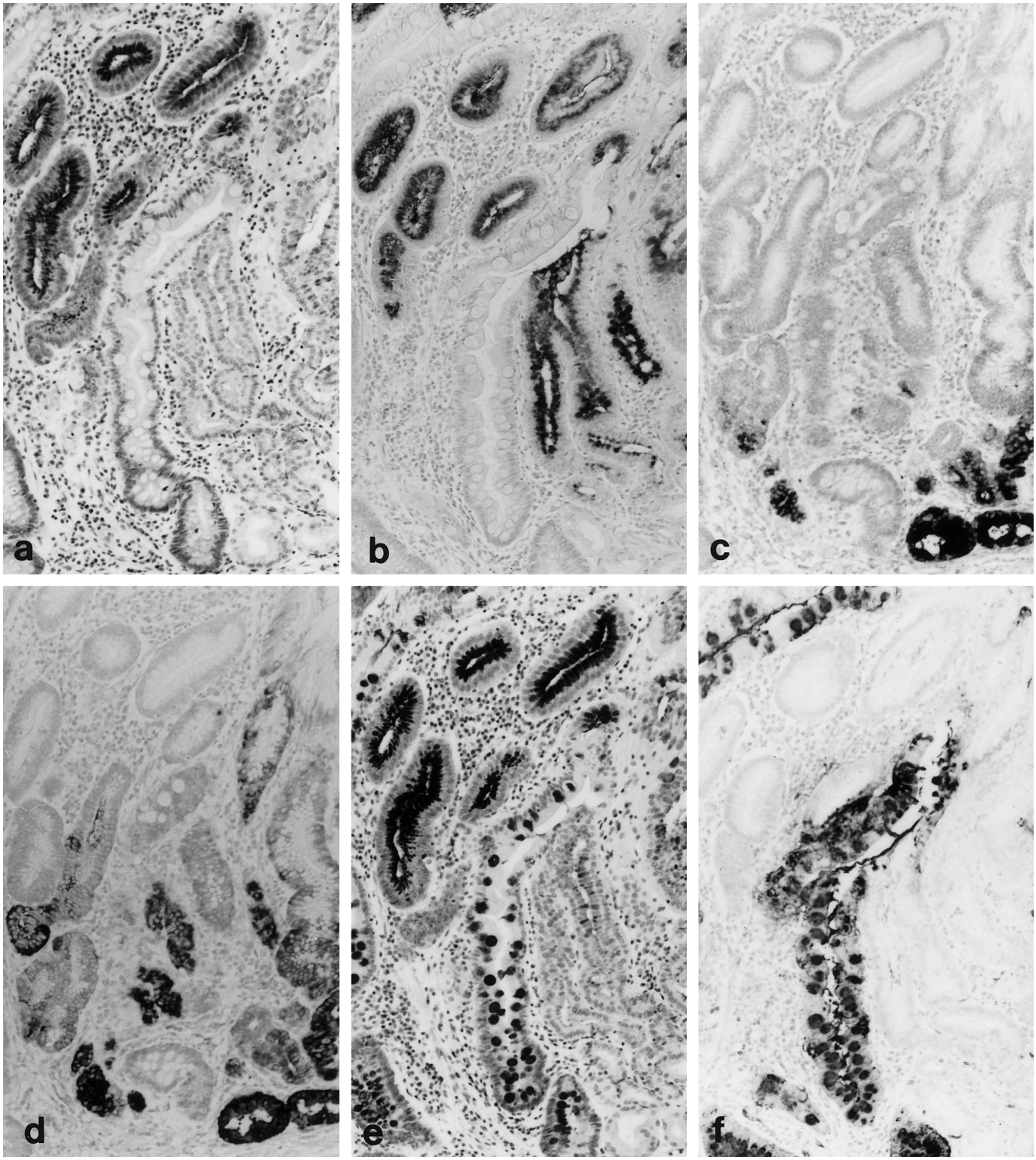

Fig. 1a–f Serial sections of surrounding pyloric mucosa demonstrating intestinal metaplasia. Surface mucous cells contain **a** galactose oxidase–Schiff (GOS)-reactive mucins, **b** SH-9 reactive mucin and **e** sialidase GOS (S-GOS)-reactive mucins. Pyloric gland cells contain class III mucins stained by **c** paradoxical concanavalin A (ConA) and **d** Pg II. Goblet cells contain **e** S-GOS-reactive mucins and **f** TKH-2-reactive mucins. **a** GOS staining, ×33; **b** SH-9 immunohistochemistry, ×33; **c** paradoxical ConA staining, ×33; **d** Pg II immunohistochemistry, ×33; **e** S-GOS staining, ×33; **f** TKH-2 immunohistochemistry, ×33

GOS- and TKH-2-positive mucin but exhibited no GOS or SH-9. Carcinoma cells of microcyst type featured intracellular microcysts that were not positive for any staining.

Hybrid cells expressing both surface mucus and goblet features were found in 15 cases of mixed phenotype carcinomas. While a few carcinoma cells of surface mucous cell type with GOS-positive mucins had no SH-9-reactive mucins, or vice versa, the proportion of carcinomas cells with GOS-positive mucins was almost as same

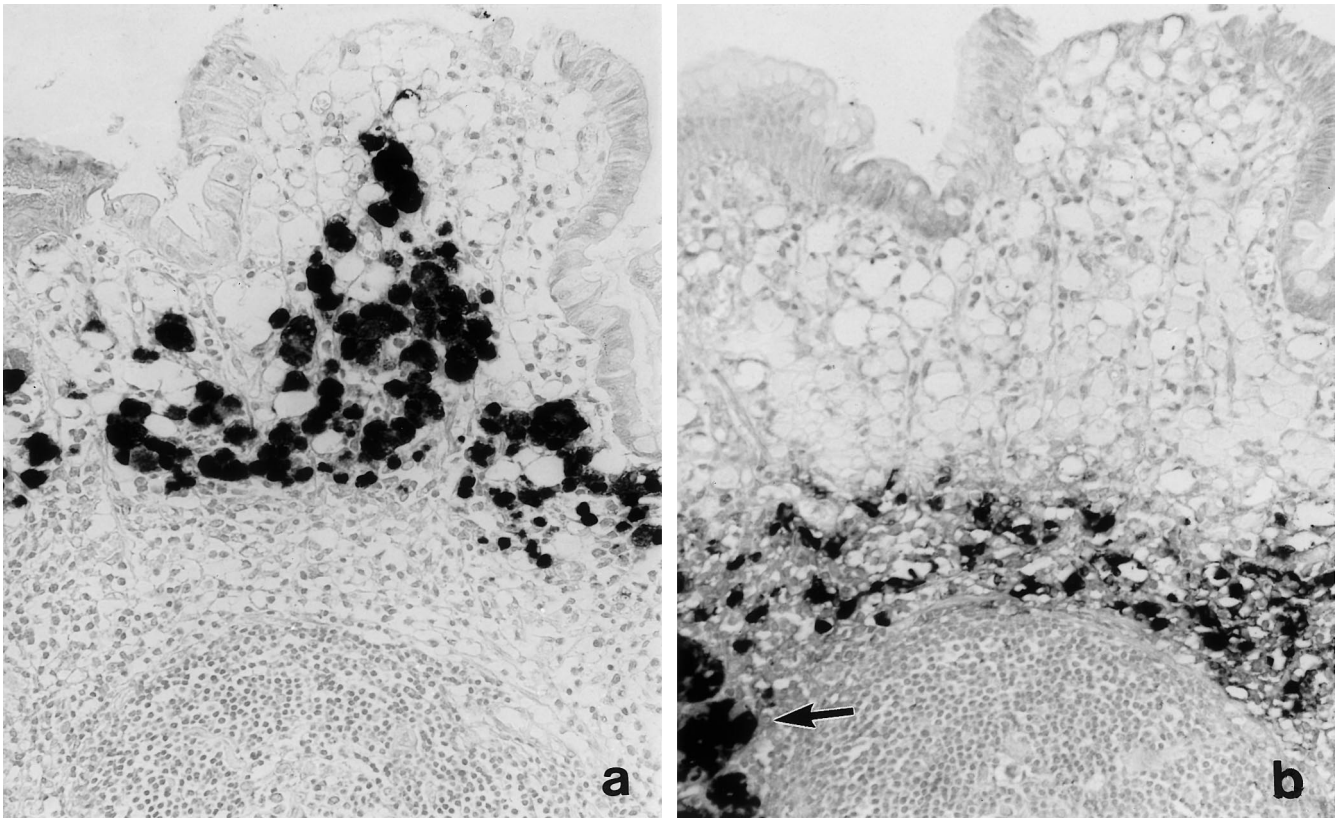

Fig. 2a, b Serial sections of an intramucosal signet ring cell carcinoma. **a** Signet ring cells of the surface mucous cell type stained by SH-9 and **b** pyloric gland cell type stained by paradoxical ConA retain a tendency for distribution in the superficial and deep parts of the mucosa, respectively. The *arrow* shows a normal pyloric gland whose cells contain class III mucins stained by paradoxical ConA. **a** SH-9 immunohistochemistry, ×50; **b** paradoxical ConA staining, ×50

as that for SH-9-reactive mucins (about 80%). Similarly, some goblet cells with S-GOS-positive mucins had no TKH-2-reactive mucins and vice versa, the proportion of carcinoma cells with GOS-negative and S-GOS positive mucins being almost the same as that for TKH-2-reactive mucins (about 60%). In the pyloric gland cell type cells, the proportion of carcinoma cells with class III mucins (about 70%) tended to be higher than that of Pg-II-positive ones (about 50%).

In the intramucosal carcinomas, cells of surface mucous cell type retained a tendency to be distributed in the superficial part and those of pyloric gland cell type in the deep part of the mucosa (Fig. 2a, b). These carcinomas were found to consist mainly of gastric epithelial cell type cells, with a few intestinal epithelial cell type cells distributed randomly, independently of the presence of intestinal metaplasia in the surrounding mucosa.

In the carcinomas invading below the submucosa, the mucosal areas showed disorganized layered structures

Table 3 Relation between phenotypic expression of signet ring cells and properties of the surrounding mucosa (*m* carcinoma limited to the mucosa, *sm* carcinoma involving the submucosa, *mp* carcinoma involving the muscularis propria, *s* carcinoma invading below the subserosa, *I.M.* intestinal metaplasia)

Depth of invasion	No. of carcinomas of each phenotype (%)						Total	
	Gastric[a]		Mixed[b]		Intestinal[c]		I.M. (+)	I.M. (–)
	I.M. (+)	I.M. (–)	I.M. (+)	I.M. (–)	I.M. (+)	I.M. (–)		
m	29 (58.0)	21 (42.0)	3 (75.0)	1 (25.0)	0	0	32 (59.3)	22 (40.7)
sm	14 (60.9)	9 (39.1)	7 (70.0)	3 (30.0)	0	0	21 (63.6)	12 (36.4)
mp	7 (58.3)	5 (41.7)	5 (62.5)	3 (37.5)	0	0	12 (60.0)	8 (40.0)
s	26 (57.8)	19 (42.2)	28 (59.6)	19 (40.4)	3 (75.0)	1 (25.0)	57 (59.4)	39 (40.6)

[a] Gastric phenotype carcinoma consisting of over 90% gastric epithelial cell type carcinoma cells

[b] Mixed phenotype carcinoma consisting of between 10% and 90% gastric and/or intestinal epithelial cell type carcinoma cells

[c] Intestinal phenotype carcinoma consisting of over 90% intestinal epithelial cell type carcinoma cells

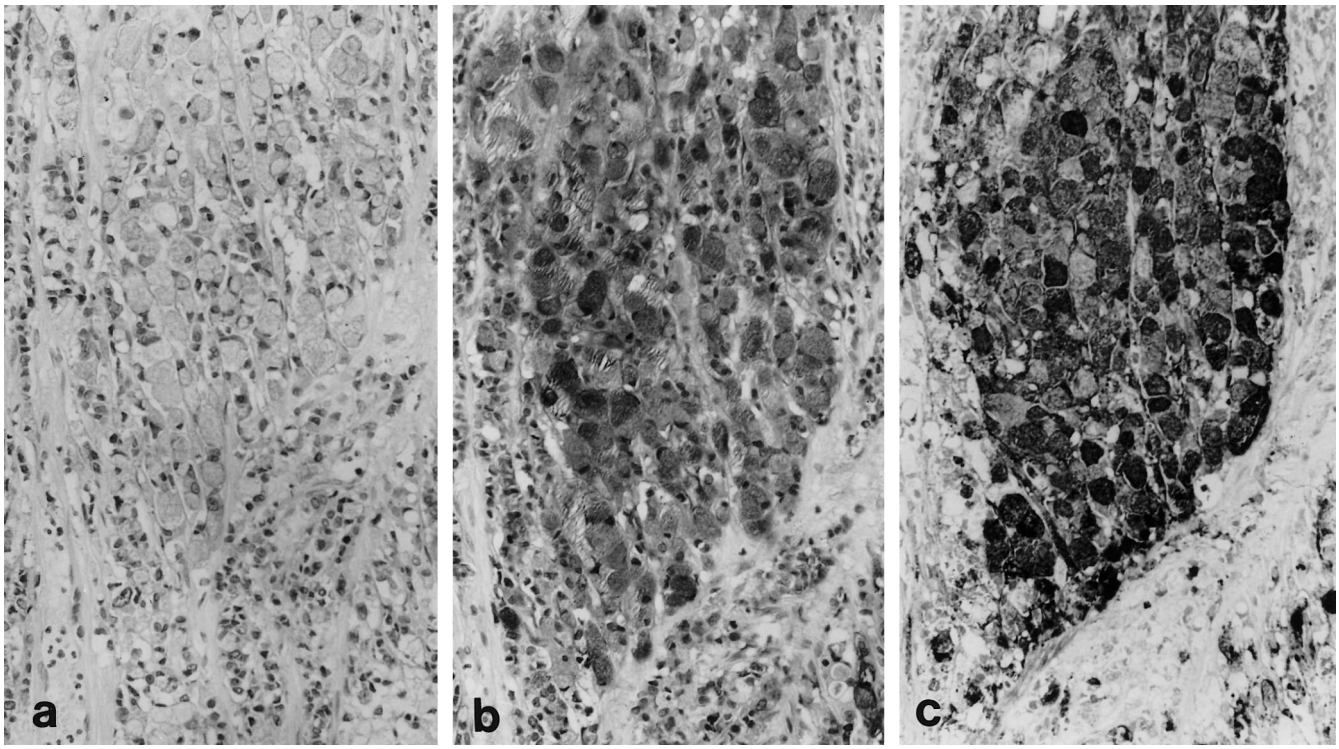

Fig. 3a–c Serial sections through an area of goblet cell type carcinoma cells in a signet ring cell carcinoma invading the serosa. Carcinoma cells which contain S-GOS (**b**) and TKH-2 reactive (**c**) but GOS-negative mucins (**a**) are randomly distributed in the subserosa. **a** GOS staining, ×50; **b** S-GOS staining, ×50; **c** TKH-2 immunohistochemistry, ×50

with each cellular phenotype being distributed at random throughout the whole tumour tissue regardless of the predominant phenotype of the carcinoma. Carcinoma cells in the subserosa of a goblet cell type carcinoma are shown in Fig. 3a–c.

The proportions of gastric, mixed and intestinal phenotypes of lesions with different depths of invasion are shown in Fig. 4. The proportion of gastric phenotype carcinomas decreased with depth of invasion, from 92.6% (involvement of the mucosa), to 69.7% (submucosa), 60.0% (muscularis propria), and 46.9% (subserosa, serosa) and that of mixed phenotype carcinomas increased with depth of invasion, from 7.4% (mucosa), to 30.3% (submucosa), 40.0% (muscularis propria), and 49.0% (subserosa, serosa). The intestinal phenotype was limited to four carcinomas involving the serosa. The decrease in gastric and increase in mixed and intestinal type carcinomas with depth of invasion were significant ($P<0.001$). A phenotypic shift from gastric to intestinal type expression was thus observed with depth of invasion of signet ring cell gastric carcinomas. The proportion of intestinal epithelial cell type carcinoma cells in mixed phenotype carcinomas tended to increase with depth of invasion (data not shown).

Results for the relation between phenotypic expression of carcinoma and that of the surrounding mucosa are summarized in Table 3. Of the 203 carcinomas , 122 (60.1%) were surrounded by intestinal metaplasia and 81

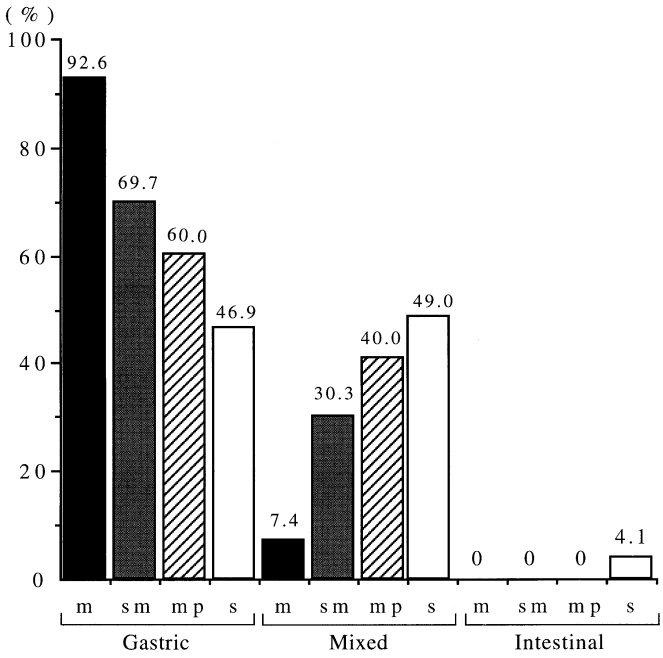

Fig. 4 Changes in phenotypic expression of signet ring cell carcinomas with progression (*m* carcinoma limited to the mucosa, *sm* carcinoma involving the submucosa, *mp* carcinoma involving the muscularis propria, *s* carcinoma invading below the subserosa, *Gastric* gastric phenotype carcinoma with over 90% gastric epithelial cell type carcinoma cells, *Intestinal* intestinal phenotype carcinoma with over 90% intestinal epithelial cell type carcinoma cells, *Mixed* mixed phenotype carcinoma with between 10% and 90% gastric and/or intestinal epithelial cell type carcinoma cells)

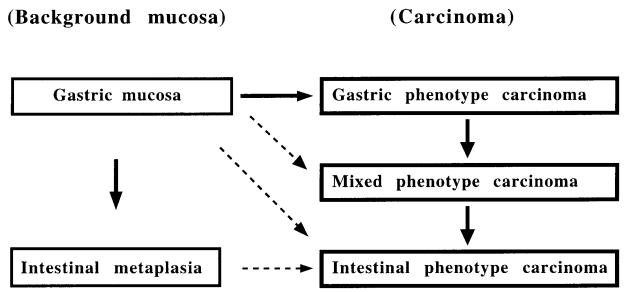

Fig. 5 Schematic illustration of pathways of gastric carcinoma genesis. The human gastric carcinomas arise primarily from the gastric mucosa, the change from gastric to mixed and to intestinal phenotype with time occurring independently of and separately from the corresponding metaplastic shift in the background mucosa. *Bold arrows* main routes, *dashed arrows* side routes

(39.9%) were observed in gastric mucosa without intestinal metaplasia. The proportions of carcinomas with intestinal metaplasia in gastric and mixed phenotypes with depth of invasion are shown in Table 3. No significant differences were found among carcinomas with intestinal metaplasia for any depth of invasion.

The proportion of gastric phenotype carcinomas in each glandular stomach region decreased and that of mixed phenotype carcinomas increased with depth of invasion, while that of carcinomas with intestinal metaplasia scarcely changed. The rate was low in the fundic gland region and high in the pyloric gland region, regardless of the depth of invasion (data not shown). Thus, no clear relationship was evident between phenotypic expression of gastric carcinoma cells and the surrounding mucosa.

Discusion

In discussions of the histogenesis of gastric cancer, it has generally been concluded that glandular type [3] (differentiated type) carcinomas arise from areas of intestinal metaplasia while the isolated cell type [3] (undifferentiated type) lesions originate in normal gastric mucosa [4, 9, 22–24]. However, a number of cases for which this theory does not apply have been reported. For example, the incidence of glandular type carcinomas in cardiac mucosa featuring a low degree of intestinal metaplasia was shown to be higher than that of isolated cell type carcinomas [16]. In another study, Tatematsu et al. showed that 33 of 122 glandular type carcinomas (27.1%) in 229 surgically obtained primary lesions consisted mainly of gastric epithelial cell type carcinoma cells [33], and Kushima et al. reported that 10 of 43 glandular type intramucosal gastric carcinomas (23.2%) had gastric phenotypic expression [20]. These findings suggest that the relationship between glandular type carcinoma and intestinal metaplasia is not intimate, and raise the question as to whether the glandular type carcinomas develop from intestinal metaplasia. We proposed earlier that the development of intestinal epithelial cell type cells in gastric epithelium and in gastric carcinomas during experimentally induced gastric carcinogenesis in rats might occur independently [29]. In human gastric carcinoma, we consider that both glandular and isolated cell type carcinomas arise primarily from the normal gastric mucosa; the change from gastric to intestinal phenotype with time occurs independently of and separately from the corresponding metaplastic shift in the background mucosa (Fig. 5). This is part of the rationale for the present study of signet ring cell gastric carcinomas.

Our results on the phenotypic expression of signet ring cells show that some tumour cells lose the phenotypic characteristics of the normal gastric epithelium, reflecting incomplete cellular differentiation. Thus, in this study, using plural markers, we were able to define cellular differentiation of carcinoma cells more clearly than previously [30–33].

With regard to phenotypic expression of signet ring cell carcinomas, in intramucosal carcinomas cells of surface mucous cell type retained a tendency to be distributed in the superficial part and those of pyloric gland cell type in the deep part of the mucosa. In advanced carcinomas the mucosal areas showed disorganized layered structures with each cellular phenotype distributed at random throughout the whole of the tissue. These results are compatible with the report that intramucosal carcinomas show organoid differentiation whereas advanced carcinomas do not [7].

The main result relating to alteration of phenotype indicated a phenotypic shift from gastric to intestinal type expression with depth of invasion. Tumour invasion depends to some extent on time, and we can therefore consider the depth of invasion as an index of the age of a carcinoma. This conclusion is compatible with the clinicopathological finding that carcinomas with mixed or intestinal phenotype tend to be larger than gastric phenotype carcinomas (data not shown). It is also compatible with our previous reports that tumour cells of gastric epithelial cell type change to intestinal epithelial cell type with growth from small to large tumours during experimentally induced gastric carcinogenesis in rats [30]. However, as the results in this study are valid only for signet ring cell carcinomas, similar studies of glandular type carcinomas are necessary to prove our hypothesis. We defined carcinomas with over 90% gastric or intestinal phenotypic expression as “purely” differentiated. If we define carcinomas with 100% gastric or intestinal phenotypic expression as “purely” differentiated, the number of gastric phenotype carcinomas will decrease and that of mixed phenotype lesions increase. The number of intestinal tumours would then decline, of course, to zero, and the results would be compatible with our conclusions.

Concerning the relation between phenotypic expression of carcinoma cells and that of the surrounding mucosa, the number of intestinal phenotype carcinomas in the present series was too small to allow conclusions. However, the present results are in line with our hypothesis. At first sight, the finding of carcinomas consisting

predominantly of gastric epithelial type cells surrounded by intestinal metaplasia might appear inconsistent with the hypothesis, but it could be explained by an origin from normal gastric mucosa and subsequent maintenance of the gastric phenotype in carcinoma tissue despite surrounding metaplasia. Conversely, carcinomas consisting predominantly of intestinal type cells surrounded by non-metaplastic glandular mucosa can be interpreted as a shift from the gastric phenotype limited to the carcinoma.

The intestinalization of gastric carcinoma could be related to the fact that all Chordata have an intestinal epithelium, but the Cyclostomes, Holocephali, some lung fishes and many Teleosts lack a morphologically distinguishable stomach [1]. Thus, intestinal gene expression is found at all levels of vertebrates, whereas the gastric phenotype only appears in higher animals. When cells lose their differentiation on treatment with various agents, they occasionally show phylogenic reversion. Intestinal metaplasia in normal pyloric mucosa might be considered to be a phylogenic reversion of gastric mucosa to intestinal mucosa. Similarly, cells of intestinal epithelial cell type in gastric carcinomas might be considered to indicate a similar process during neoplasia [30].

In conclusion, the present study provides evidence that the phenotype of signet ring cell gastric carcinomas changes from gastric epithelial to intestinal epithelial type with progression.

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