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Gastric phenotypic expression in human gallbladder cancers revealed by pepsinogen immunohistochemistry and mucin histochemistry

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Summary. Gastric phenotypic expression indicated by paradoxical concanavalin A (Con A) staining for class III mucins and the immunoperoxidase method for pepsinogen (Pg) I and Pg II was found in pyloric gland metaplasia of gallbladder epithelium. Using the same methods, the features of gallbladder cancers and their relationship to pyloric gland metaplasia in the human gallbladder epithelium were studied. Histologically, 57 gallbladder cancers were classified into 5 papillary adenocarcinomas, 29 tubular adenocarcinomas, 8 poorly differentiated adenocarcinomas, 6 signet-ring cell carcinomas, 4 mucinous adenocarcinomas, and 5 squamous cell carcinomas. In papillary and tubular adenocarcinomas, Pg I and/or Pg II staining was detected in 80% and 75.9% of cancers, respectively. Pg II staining was significantly more frequent than Pg I staining. One signetring cell carcinoma also had Pg II activity. Pyloric gland metaplasias all contained class III mucins and were further classified into complete type and incomplete type on the basis of presence or absence Pg I and/or Pg II activities. A few cancer cells with class III mucins were negative for Pg staining; conversely, a few cells with Pg I and/or Pg II had no class III mucins. Phenotypic diversity in both class III mucin reactivity and Pg activities was observed in gallbladder cancer cells with the pyloric gland cell type. By comparison, pyloric gland metaplasia varied only in Pg activities. A few Pg-positive cancers were found in the gallbladder with Pg-negative pyloric gland metaplasia. The present results clearly indicate the appearance of gastric phenotypic expression in both gallbladder epithelium and gallbladder cancers and suggest the independent induction of pyloric gland metaplasia and cancer with gastric phenotypic expression.

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Introduction

Human pepsinogens have been classified into 2 groups, namely pepsinogen (Pg) I and Pg II, which can be detected immunohistochemically in pyloric gastric mucosa (Samloff 1969 and 1971; Ichinose et al. 1982). Paradoxical concanavalin A (Con A) staining enables histochemical classification of mucins in the alimentary tract into 3 main classes (I, II, and III) and normal gastric pyloric gland cells contain class III mucins (Katsuyama and Spicer 1978; Tatematsu et al. 1980). According to the immunohistochemical reaction for Pgs I and II and class III mucin reactivity, pyloric gland metaplasia of two types has been described in the gallbladder epithelium (Tatematsu et al. 1987b): The complete type of pyloric gland metaplasia contains class III mucins and both pepsinogen activities, like normal pyloric gland cells. The incomplete type of pyloric gland metaplasia contains class III mucins, but no Pg I or II activity.

In this study, we investigated gastric phenotypic expression in gallbladder cancers and in surrounding gallbladder epithelium using immunohistochemical methods for Pgs I and II and paradoxical Con A staining for mucins.

Materials and methods

Samples of primary gallbladder cancers (14 surgical, 43 autopsy) and stomach (10 surgical) were examined. All specimens were fixed in 10% buffered formalin and embedded in paraffin. Preparations were stained histochemically for mucins with Alcian blue (AB) (pH 2.5), PAS, and paradoxical Con A staining (Katsuyama and Spicer 1978). Mucins (mucoproteins) which

Table 1. Age and sex of patients having gallbladder cancer and their histologic types

Age range	Histologic type											
	Papi.		Tubular		Poorly		Signet		Mucinous		Squamous	
	M	F	M	F	M	F	M	F	M	F	M	F
21–30							1					
31–40		1										
41–50			1	1				1			1	
51–60			2	4	1	1			1			2
61–70	1	2	3	10	1	2	1	2	1	1		
71–80			1	4	1	2	1			1	1	1
81–90	1		1	2								

Papi., papillary adenocarcinoma; Tubular, tubular adenocarcinoma; Poorly, poorly differentiated adenocarcinoma; Signet, signet-ring cell carcinoma; Mucinous, mucinous adenocarcinoma; Squamous, squamous cell carcinoma; M, male and F, female

are stained with Con A (Wako Pure Chemical Industries, Ltd., Osaka, Japan)-HRP, mostly lose their stainable properties after oxidation with periodate. However, some mucins showed paradoxical enhancement of staining after periodate oxidation with and without subsequent reduction (Re) or blockage of oxidized groups. By this paradoxical Con A staining, mucins in the alimentary tract were classified into 3 main classes (classes I to III) and the procedure of paradoxical Con A staining, periodic acid (PA)-reduction (Re)-Con A-HRP was used in this study. Class III mucins (brown by PA-Re-Con A-HRP) which were found only in cardiac gland cells, mucous neck cells, pyloric gland cells and Brunners' gland cells could be distinguished from class II (unstained by PA-Re-Con A-HRP) mucins.

The ABC method (Hsu et al. 1981) was used to demonstrate the immunohistochemical location of Pg I and Pg II. Anti Pg I and Pg II antibodies were prepared as described previously (Ichinose et al. 1982). Affinity purified biotin-labelled goat anti-rabbit immunoglobulin IgG and the avidin-biotin-peroxidase complex (Vectastain ABC Kit, PK 4001) were obtained from Vector Laboratories (Burlingame, CA, USA). Sections were deparaffinized routinely by passage through petroleum benzene and a graded alcohol series and then treated sequentially with normal goat serum, rabbit anti Pg I (1:4000) or Pg II (1:4000), biotin-labelled goat anti rabbit IgG (1:400), and avidin-biotin-peroxidase complex (ABC). The site of peroxidase binding was detected by the diaminobenzidine method of Graham and Karnofsky (1966). Sections were counter stained with haematoxylin for microscopic examination. As a negative control for the specificity of anti-Pg I or Pg II antibodies, preimmune rabbit serum was used instead of each antiserum.

Cells containing class III mucins and/or pepsinogens were considered to indicate phenotypic expression of pyloric gland cells. The presence of mucous cells containing class III mucins in gallbladder epithelium was defined as pyloric gland metaplasia. Tumour cells containing class III mucins and/or pepsinogens were considered to indicate phenotypic expression of pyloric gland cells.

Results

Of the 57 gallbladder cancer cases, 18 were men, and 39 were women; they ranged in age from 28 to 87 years (mean, 63.4 years). The age and sex of patients bearing the gallbladder cancers and their histological types are summarized in Table 1.

On paradoxical Con A staining (PA-Re-Con A-HRP procedure), class III mucins were found in all pyloric gland cells. On immunohistochemical staining of pyloric gland cells, Pg II stained moderately positive. However, Pg I was not detectable except for occasional glands in the deeper part of the mucosa.

Surrounding non-neoplastic gallbladder epithelium was examined in 34 out of 57 cases. In the others, surrounding gallbladder epithelial layers were replaced by cancer cells. No class III mucins or Pgs I and II were found in normal looking gallbladder epithelium. Pyloric gland metaplasia of surrounding epithelium was found in 33 of 34 cases. Pyloric gland metaplasias were further classified into a complete type and an incomplete type. The complete type of pyloric gland metaplasia (positive for Pg II and occasionally for Pg I, like normal pyloric gland cells of gastric mucosa) was found frequently (25/34; 73.5%). The incomplete type of pyloric gland metaplasia (Fig. 1a–c) (negative for Pgs I and II) was found in all gallbladders with pyloric gland metaplasia.

Of the 57 carcinomas studied, 5 were papillary adenocarcinomas, 29 were tubular adenocarcinomas, 8 were poorly differentiated adenocarcinomas, 6 were signetring cell carcinomas, 4 were mucinous carcinomas and 5 were squamous cell carcinomas. Table 2 shows the results of the immunohistochemistry for Pgs I and II and paradoxical Con A staining for mucins of each histological variant. Pg- and class III mucin-positive cancer cells were found significantly ($P<0.01$) more frequently in papillary and tubular adenocarcinomas (Fig. 2a–c). Of the other histological types, one signet-ring cell carcinoma only contained class III mucins and Pg II (Fig. 3a–c). Although all Pg-positive cancer cells and Pg II activity, only a few

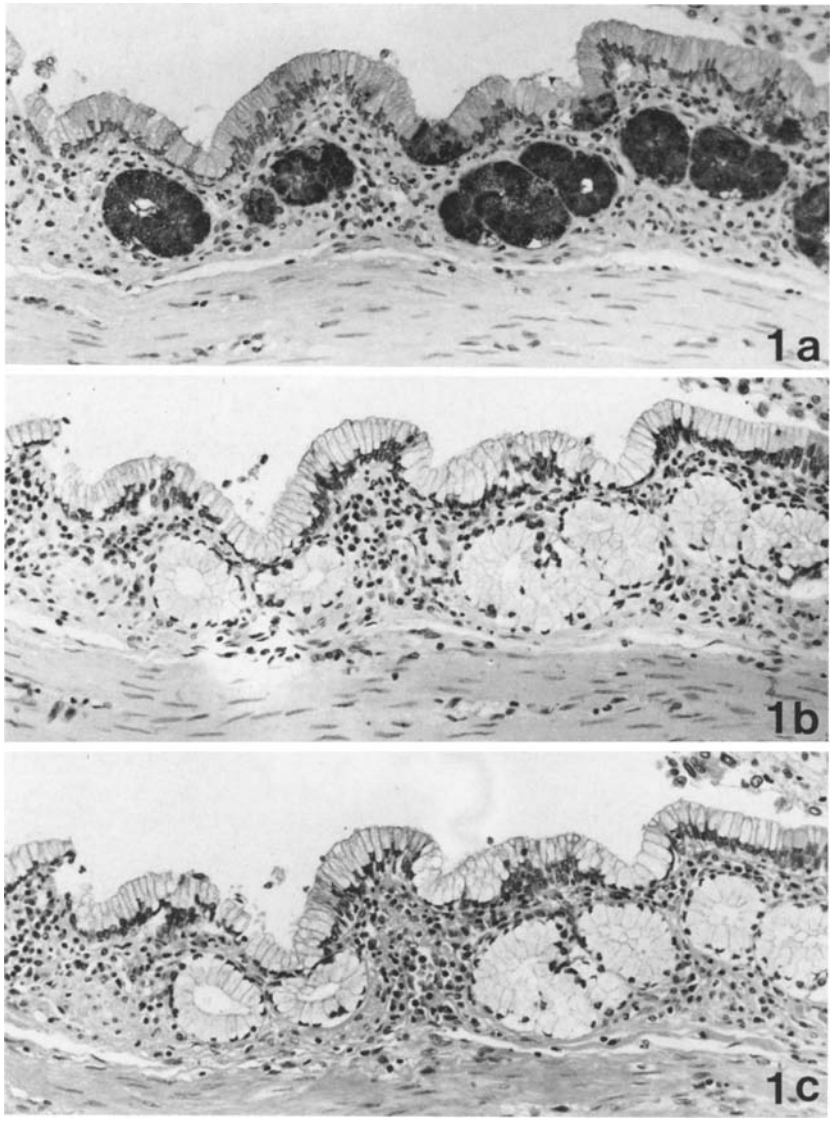

Fig. 1a–c. Incomplete type pyloric gland metaplasia of the gallbladder. All pyloric gland type metaplastic cells contained class III mucins **a**, no Pg I **b** and no Pg II **c**. **a**; paradoxical Con A staining ×200, **b**; Pg I immunostaining, ×200, **c**; Pg II immunostaining, ×200

Table 2. Gastric phenotypic expression of gallbladder carcinomas

Histology	No. of case	No. of cases showing gastric phenotypic expression			
		Pg I	Pg II	Class III	Neither
Papillary adenocarcinoma	5	2	4	3	1
Tubular adenocarcinoma	29	3	22	19	7
Poorly differentiated adenocarcinoma	8	0	0	0	8
Signet-ring cell carcinoma	6	0	1	1	5
Mucinous adenocarcinoma	4	0	0	0	4
Squamous cell carcinoma	5	0	0	0	5
Total	57	5	27	23	30

cells had Pg I activity. No cancers composed of Pg I-positive and Pg II-negative cells were found. Although class III mucins were always found in pyloric gland metaplasia, Pg-positive cancer cells occasionally showed no class III mucin reactivity; conversely, a few cancer cells with class III mucins had no Pg I and Pg II. Class III mucin-positive cancers all contained Pg II positive cancer cells. However, no cancer cells with class III mucins were found in 3 out of 27 Pg II-positive cancers. Gastric

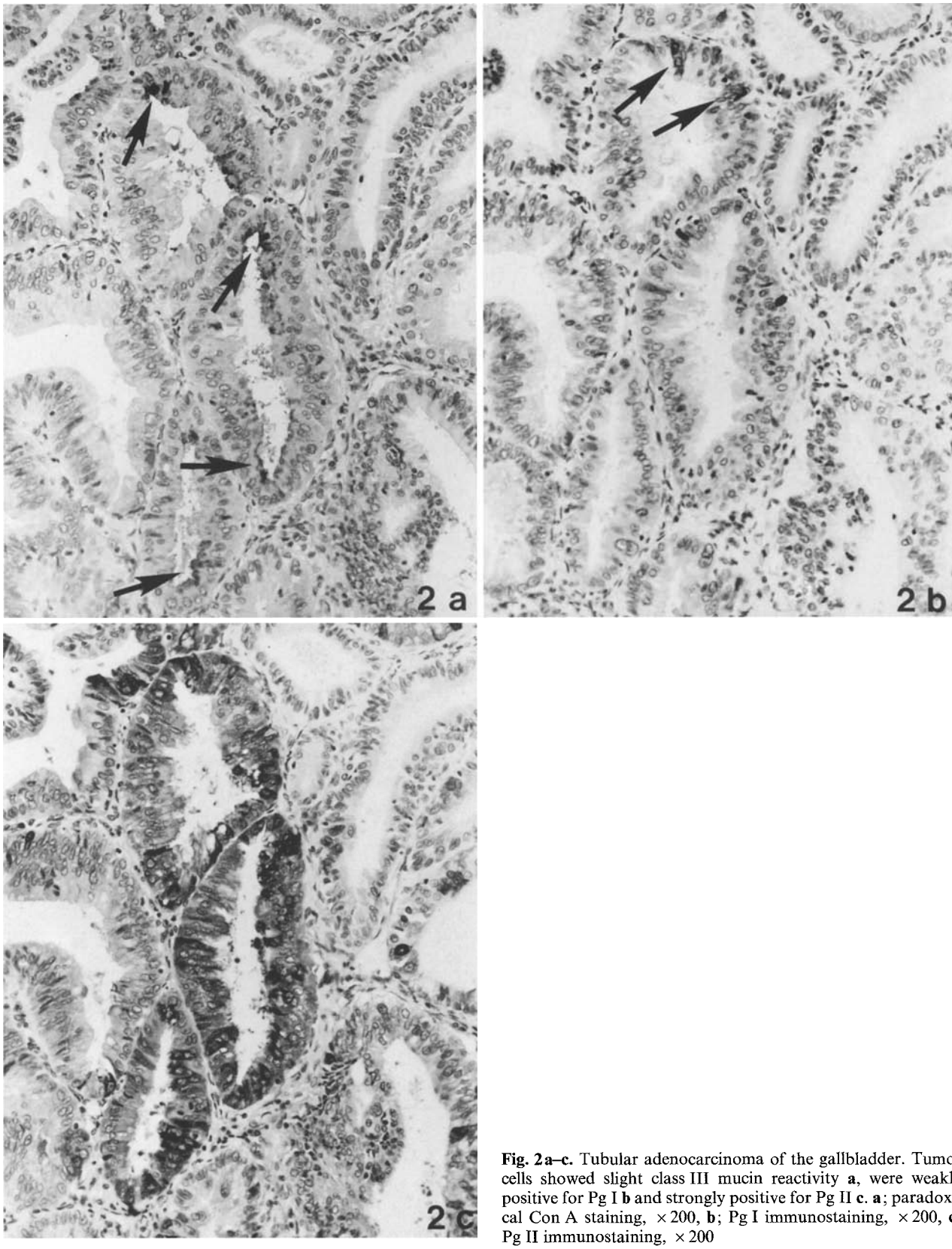

Fig. 2a–c. Tubular adenocarcinoma of the gallbladder. Tumor cells showed slight class III mucin reactivity **a**, were weakly positive for Pg I **b** and strongly positive for Pg II **c**. **a**; paradoxical Con A staining, ×200, **b**; Pg I immunostaining, ×200, **c**; Pg II immunostaining, ×200

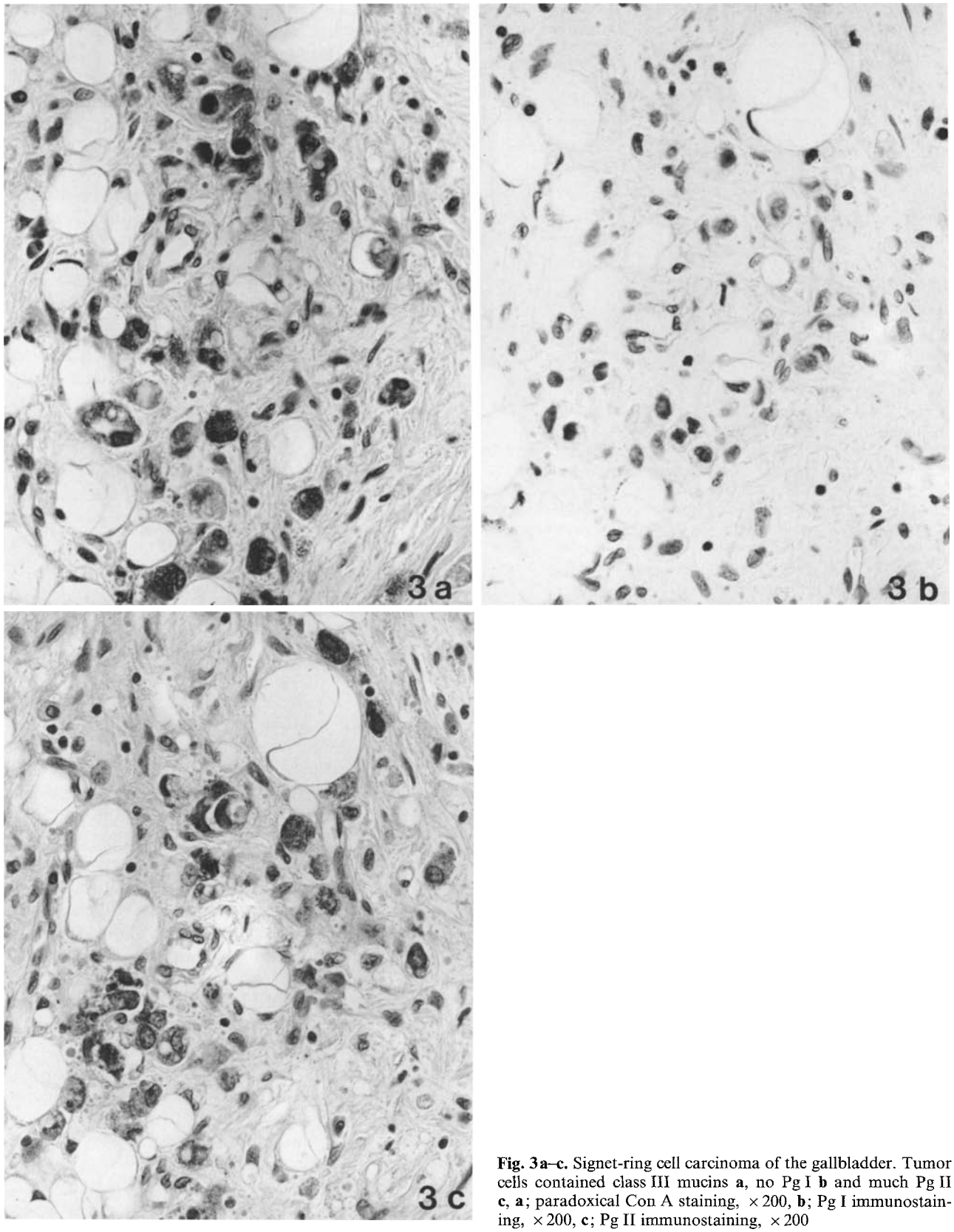

Fig. 3a–c. Signet-ring cell carcinoma of the gallbladder. Tumor cells contained class III mucins **a**, no Pg I **b** and much Pg II **c**, **a**; paradoxical Con A staining, ×200, **b**; Pg I immunostaining, ×200, **c**; Pg II immunostaining, ×200

Table 3. Relation of pepsinogen (Pg) and class III mucin production to pyloric gland metaplasia and gallbladder cancers

Surrounding gallbladder epithelium	No. of pepsinogen positive cancers			
	Pg I	Pg II	Class III	Neither
Papillary adenocarcinoma				
Complete	2	4	3	0
Incomplete	0	0	0	1
Tubular adenocarcinoma				
Complete	2	13	12	2
Incomplete	1	3	2	1
No metaplasia	0	0	0	1
Signet-ring cell carcinoma				
Complete	0	1	1	0
Incomplete	0	0	0	0
Poorly differentiated and mucinous adenocarcinoma				
Complete	0	0	0	3
Incomplete	0	0	0	4

Thirty four cases of gallbladder cancers with surrounding non-neoplastic gallbladder epithelium were analyzed. Pg I and/or class III mucins positive cancer cells all were found in cancers containing Pg II positive cells. Pg I and Pg II, pepsinogens I and II; class III, class III mucins; complete, complete type of pyloric gland metaplasia; Incomplete, incomplete type of pyloric gland metaplasia

phenotypic expression of cancer cells appeared not to be related to the stage of cancer but to histological type, since early and/or advanced stages of papillary or tubular adenocarcinomas showed gastric phenotypic expression but early and/or advanced stages of poorly differentiated adenocarcinomas indicated no gastric phenotypic expression.

The results of pepsinogen and class III mucin staining of cancer cells of each histological type and of pyloric gland metaplasias in surrounding gallbladder epithelium are summarized in Table 3. Pg- and/or class III mucin-positive cancer cells mainly developed in gallbladder epithelium with Pg-positive pyloric gland metaplasia. However, Pg-positive cancer cells occasionally developed in gallbladder epithelium with incomplete pyloric gland metaplasia (Pg-negative). Pg-negative cancer cells were sometimes found in gallbladder epithelium with complete type (Pg-positive) pyloric gland metaplasia. Class III mucin negative and Pg positive cancer cells occasionally developed in gallbladder epithelium with complete and/or incomplete type pyloric gland metaplasias.

Discussion

The generation of specific antisera to human Pgs I and II (Samloff 1971; Samloff and Liebman 1973; Ichinose et al. 1982; Tatematsu et al. 1987b) and rat Pg I (Furihata et al. 1976 and 1980; Tatematsu et al. 1987a) has provided the means of immunohistochemical description of Pg distribution in normal and diseased tissues. Pg I production occurs principally in chief cells and mucous neck cells of the gastric fundic glands and occasionally in pyloric gland cells, while Pg II is distributed in chief cells, mucous neck cells, and pyloric gland cells throughout the fundic and pyloric glands. Class III mucins are found in cardiac gland cells, mucous neck cells, pyloric gland cells of the stomach and also in Brunner's gland cells throughout the mammalian alimentary tract (Katsuyama and Spicer 1978; Tatematsu et al. 1980). In comparative studies on the gastric mucosa of various vertebrates, pyloric glands were found in the stomach of amphibians, reptiles, and mammals. These pyloric gland cells all contain class III mucins (Suganuma et al. 1981). In addition, class III reactivities are retained under pathological conditions and have been shown to be good markers of the cell type of human and rat gastric cancers (Tatematsu et al. 1980; Furihata et al. 1984). Class III mucin staining has been considered as a stable marker of pyloric gland cells. Thus, gastric phenotypic expression of gallbladder epithelium in pyloric gland metaplasia and gallbladder cancer cells was clearly indicated by paradoxical Con A staining and immunohistochemistry of pepsinogens.

Cells of pyloric gland metaplasia in stomach and gallbladder all contain class III mucins (Tsutsumi et al. 1984; Tatematsu et al. 1987b). Pyloric gland metaplasia was previously classified into complete type with Pgs I and II, incomplete type 1 with Pg II but no Pg I and incomplete type 2 with no Pgs I and II (Tatematsu et al. 1987b). However, with a 1:1200 dilution of Pg I antiserum, Pg I reactivity in normal pyloric gland of gastric mucosa was evident, although only a few pyloric gland cells are Pg I-positive (Meuwissen et al. 1984). In this study, we used a 1:4000 dilution of Pg I antiserum, which enabled us to classify clearly pyloric gland metaplasias into complete type (Pgs I and II, or Pg II only) and incomplete type with no Pg activity.

Class III mucin reactivity is stable in pyloric gland metaplasia (Tatematsu et al. 1987b). Phenotypic alteration appeared only in Pg activities of cells in pyloric gland metaplasias. However, a few gallbladder cancer cells with class III mucins had no Pg I and Pg II; conversely, a few with Pg I and/or Pg II had no class III mucins. Phenotypic changes in gallbladder cancer cells with the pyloric gland cell type involved both class III mucin reacti-

vities and Pg activities. Cancer cells that contained neither class III mucins nor pepsinogens appeared more altered than tumour cells containing both class III mucins and Pgs. Gallbladder cancers consisting of phenotypically more altered cells might be reasonably considered to be less differentiated.

Similar to intestinal metaplasia in the stomach, the relation of intestinal metaplasia in the gallbladder to carcinoma recently has been drawing the attention of some investigators (Hirai 1980; Yamagiwa and Tomiyama 1986; Albores-Saavedra et al. 1986). Intestinal phenotypic expression of gallbladder cancer cells as well as intestinal metaplasia in its surrounding mucosa have both been observed. However, intestinal metaplasia is less frequent than pyloric gland metaplasia (Tsutsumi et al. 1984; Yamagiwa and Tomiyama 1986). In our studies, phenotypic expression of pyloric gland type cells was more frequently found at rates of 80% and 75.9% in papillary and tubular carcinoma cells. Thus, pyloric gland metaplasia should be considered to be a more important preneoplastic change leading to gallbladder carcinoma.

In previous work, we showed that intestinal metaplasia and the appearance of cells of intestinal type in adenocarcinomas occur independently during experimental gastric carcinogenesis in rats (Tatematsu et al. 1983). Also human diffuse-type carcinomas which originated from nonmetaplastic gastric mucosa (Nakamura et al. 1968) consisted of intestinal and/or gastric type cells (Furihata et al. 1984; Tatematsu et al. 1986). As intestinal phenotypic expression of gastric cancer cells is stable (Tatematsu et al. 1984), the intestinal type cell component of human gastric cancers probably increases with time. In the gallbladder, squamous cell metaplasia is extremely rare in the non-neoplastic mucosa, but it occurs frequently in advanced cancers (Kozuka et al. 1982). Accordingly, phenotypic metaplasia in non-neoplastic epithelium may be independent of the phenotypic changes in cancer cells. Pg-positive or negative cancers were found in the gallbladder with pyloric gland metaplasias, irrespective of Pg phenotype of surrounding metaplasia. Appearance of Pg-positive cells in cancers and pyloric gland metaplasia of gallbladder epithelium may also occur independently during gallbladder carcinogenesis. Further studies of early gallbladder cancers are needed to clarify these relationships.

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