Co-expression of gastric and biliary phenotype in pyloric-gland type adenoma of the gallbladder: Immunohistochemical analysis of mucin profile and CD10

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Abstract. Pyloric-gland type adenoma of the gallbladder is formed by proliferation of glands resembling pyloric glands, morphologically. No previous report has described the cellular phenotype and differentiation of pyloric-gland type adenoma of the gallbladder, using CD10 as a marker of proper biliary phenotype. Immunostainings were performed for mucin markers such as MUC5AC, human gastric mucin (HGM) for gastric foveolar type epithelium, MUC6, M-GGMC-1 for pyloric-gland type and MUC2 for intestinal goblet-cell type, and for CD10 as a proper biliary type marker on 58 pyloric-gland type adenomas of the gallbladder, as well as for p53, Ki-67 and CDX2. The percentage (X) of reactive cells in relation to the total number of tumor cells was estimated semi-quantitatively, and divided into four categories: X=0% (negative), 0%<X<10%, 10%≤X<30%, and X≥30%. CDX2 expression was considered to be positive when the percentage of positively stained cells was ≥10%. Out of the 58 pyloric-gland type adenomas, ≥30% of adenoma cells were positive for MUC5AC in 22 (38%) tumors, HGM in 29 (50%), MUC6 in 58 (100%), M-GGMC-1 in 54 (93%), MUC2 in none (0%), and CD10 in 20 (34%). MUC6 (P<0.001) and M-GGMC-1 (P<0.001) mucins were detected more frequently in pyloric-gland type adenomas, and CD10 expression was significantly decreased, compared with normal gallbladder epithelium (P=0.006). P53 overexpression was not found in any of the 58 tumors, including two adenomas with carcinomatous foci.

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The mean number of Ki-67-positive cells was 10.3±5.8%. CDX2 expression was judged as negative in all 58 pyloric-gland type adenomas. In pyloric-gland type adenomas of the gallbladder, expression of pyloric-gland type mucins was observed with a high frequency, whereas intestinal goblet-cell mucins were rarely seen. In addition, co-expression of gastric foveolar type mucins and CD10 was also demonstrated. Pyloric-gland type adenomas of the gallbladder show a differentiation toward pyloric glands in terms of immuno-histochemistry, as well as morphology, accompanied by co-expression of gastric foveolar and native biliary phenotypes.

Introduction

Although adenoma of the gallbladder is a rare benign tumor, it has been established as an important precursor lesion that plays a part in gallbladder carcinogenesis (1,2). According to its growth and structural pattern, it is divided into three subtypes: tubular, papillary and tubulopapillary, and it is also classified histogenetically as pyloric gland, intestinal and biliary types (1,2). Among these, the most common form is tubular adenoma of pyloric gland type (1,3), which is typically present as well-demarcated, polypoid lesions in the gallbladder. Histologically, these tumors are composed of lobules with closely packed glands that resemble pyloric glands, some of which are cystically dilated (1,2). Pyloric-gland type adenoma of the gallbladder usually displays a range of epithelial dysplastic changes, often co-existing with hyperplastic and metaplastic lesions, giving rise to considerable confusion in diagnosis (1,3).

Mucins are a group of high-molecular-weight glycoproteins consisting of a mucin core protein (apomucin) and many O-linked carbohydrate side chains (4-6). Previous progress in immunohistochemistry has suggested a unique role for mucin markers based on their tissue- and cell-specific expression and distribution patterns in individual organs (7). To date, a series of human mucin genes have been assigned to the MUC gene family that are approved by the Human Genome Organization Gene Nomenclature Committee (HUGO/GNC;

Table I. Immunohistochemical markers used for determining the phenotype of gallbladder epithelium.

	Gastric foveolar type		Pyloric-gland type		Intestinal goblet-cell type	Proper biliary type
	MUC5AC	HGM	MUC6	M-GGMC-1	MUC2	CD10
Antibody clone	CLH2	45M1	CLH5	HIK1083	Ccp58	56C6
Specificity	Human Muc-5AC glycoprotein	Peptide core of human gastric mucin	Human Muc-6 glycoprotein	α-linked GlcNAc residue	Human Muc-2 glycoprotein	CD10 glycoprotein
Positive cells in non-neoplastic gallbladder epithelium	Mucous epithelium predominantly at the superficial site of crypt-like structure in normal mucosa	Mucous epithelium predominantly at the superficial site of crypt-like structure in normal mucosa	Mucous epithelium predominantly at the bottom site of crypt-like structure in normal mucosa, metaplastic pyloric type epithelium	Metaplastic pyloric type epithelium with grandular structure, including neck glands	Metaplastic epithelium of goblet-cell type	Proper biliary epithelium
Staining pattern	Intracytoplasmic	Intracytoplasmic and luminal membranous	Intracytoplasmic	Intracytoplasmic and luminal membranous	Intracytoplasmic	Apical at the striated border and rarely intracytoplasmic
Working dilution	1:100	1:50	1:100	1:50	1:500	1:200
Source	Novocastra, Newcastle, UK	Novocastra, Newcastle, UK	Novocastra, Newcastle, UK	Kanto Chemical, Tokyo, Japan	Novocastra, Newcastle, UK	Novocastra, Newcastle, UK

http://www.gene.ucl.ac.uk/nomenclature/) (8). Because the genes belonging to the MUC series express their associated proteins, they have been accepted as valid immunohistochemical markers for determining cellular phenotypes in both neoplastic and non-neoplastic lesions in various organs. Among these, MUC2, MUC5AC and MUC6 genes are clustered within the 11p15 locus (9), classified as secretory-type mucins, and specifically express the mucins associated with intestinal goblet cell, gastric foveolar and pyloric-gland type adenomas, respectively (10-15).

In pyloric-gland type adenoma of the gallbladder, some authors have analyzed their mucin profile by using immunohistochemical markers of the MUC series (8,16). In the present study, we describe the systematic analysis of the mucin profile of pyloric-gland type adenoma of the gallbladder, using a series of mucin markers; MUC5AC, human gastric mucin (HGM), MUC6, M-GGMC-1 and MUC2, and CD10 as a proper biliary type marker. In addition, we also investigated the potential for intestinal differentiation of the adenomas by immunohistochemical staining for CDX2, a caudal-related homeobox gene that plays an important role in the regulation of cell proliferation and differentiation in the gut, or intestinal metaplasia in other organs (17-19), in an attempt to better understand the cytological nature of this tumor.

Materials and methods

Materials. Out of the 13,223 gallbladders, all of which were examined at the Division of Molecular and Diagnostic Pathology, Graduate School of Medical and Dental Science, Niigata University, Japan, between 1981 and 2003, 81 (0.6%) gallbladder adenomas (78 of pyloric gland type, three of intestinal type, and none of biliary type) were obtained for the present study. Because intestinal type adenomas were extremely rare and small in number, only 78 pyloric-gland type adenomas from 59 patients were assessed clinicopathologically.

After all the resected specimens were routinely fixed in 10% buffered formalin, the size and gross type of tumors were determined by macroscopic examination. Consecutive, 4 mm-wide sections were cut and embedded in paraffin-blocks. Serial 3 μm-thick hematoxylin and eosin (H&E)-stained sections were cut and examined microscopically.

Immunohistochemistry. Out of 78 pyloric-gland type adenomas of the gallbladder, paraffin-embedded tissue samples were available for immunohistochemical staining from 58 adenomas. Nine serial sections were obtained from the representative paraffin block of each tissue sample. After deparaffinization, the tissue sections were heated by 20-min microwave treatment, and allowed to cool to room temperature. Nonspecific binding was blocked with normal serum (Nichirei, Tokyo, Japan). The tissue sections with the primary antibodies were incubated overnight at 4˚C, and then with biotinylated secondary antibodies (Nichirei) for 30 min at room temperature, followed by treatment with the streptavidin-biotinylated peroxidase complex reagent (Nichirei). Immunohistochemical staining was performed following the manufacturer's instructions. Six specific immunohistochemical markers used in this study are listed in Table I. Immunohistochemical staining for the same series of markers as above was also performed on another six gallbladders without neoplasms or severe inflammation, to compare the immunohistochemistry of 58 pyloric-gland type adenomas with normal gallbladder epithelium. To evaluate the presence of mutated inhibitory oncogene and the proliferative activity of tumor cells, immunostaining for p53 and Ki-67 was performed by the streptoavidin-biotin method using monoclonal antibody PAb1801 (p53) and monoclonal antibody MIB-1 (Ki-67), respectively. In addition, to investigate the potential for intestinal differentiation of tumor cells, CDX2 immunostaining was performed using monoclonal antibody to CDX2 protein. The antibodies to p53, Ki-67 and CDX2 used in this study and the immunohistochemical methods are summarized

Table II. Summary of antibodies to p53, Ki-67 and CDX2 and immunohistochemical methods.

Antigen	Clone	Specificity	Dilution	Antigen retrieval	Source
p53	PAb1801	p53 protein	1:400	Microwave	Oncogene Science, Cambridge, MA, USA
Ki-67	MIB-1	Ki-67 protein	1:100	Autoclave	Immunotech, Marseille, France
CDX2	CDX2-88	CDX2 protein	1:200	Microwave	BioGenex, San Ramon, CA, USA

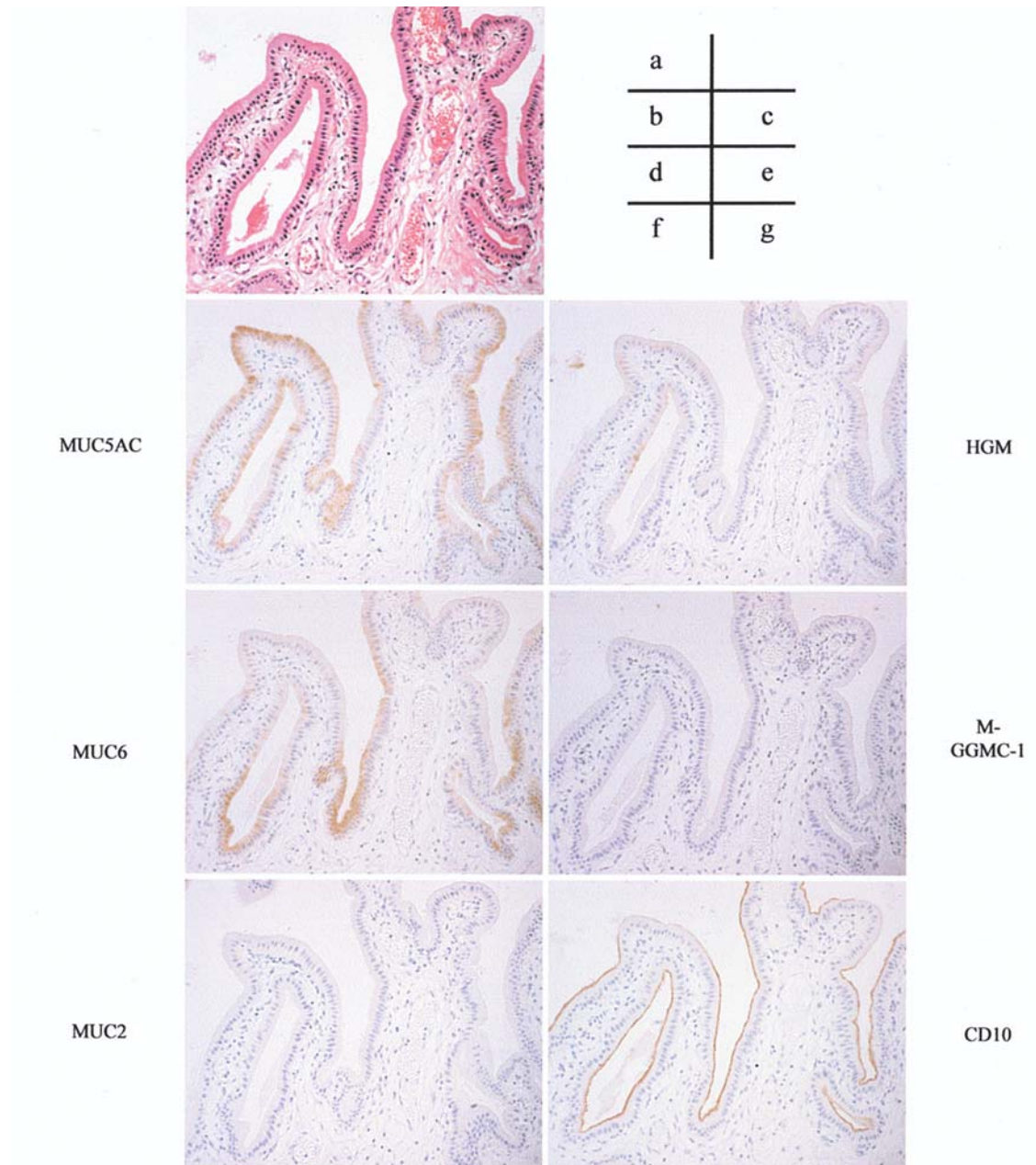
Figure 1. Immunohistochemical staining of proper gallbladder epithelium: (a) H&E section, (b) MUC5AC, (c) HGM, (d) MUC6, (e) M-GGMC-1, (f) MUC2 and (g) CD10 (x200).

in Table II. Light counterstaining was performed with Mayer's hematoxylin in all the immunostaining experiments.

Analysis of immunostaining for MUC5AC, HGM, MUC6, M-GGMC-1, MUC2 and CD10. The five specific mucin markers and CD10 were used to determine the phenotypes of 58

pyloric-gland type adenomas: MUC5AC and HGM for gastric foveolar type, MUC6 and M-GGMC-1 for pyloric-gland type, MUC2 for intestinal goblet-cell type, and CD10 for proper biliary type. The reactive sites of individual cells in non-neoplastic gallbladders are also demonstrated in Table I (Fig. 1). We considered expression in the cytoplasm as

Table III. Clinicopathological features of 78 pyloric-gland type adenomas of the gallbladder.

Patients/no. of tumors	59/78[a]
Age, years	63.8±2.7 (39-86)[b]
Sex (M/F)	18/41
Prevalence of complicating cholelithiasis	69% (33/48)
Location (Gf/Gb/Gn)[c]	15/37/23[d]
Size, mm	1.0-40.0 (mean, 9.8; median, 6.0)

[a]Six women and three men had a double lesion of adenoma, and five women had a triple lesion. [b]Values are means ± SD followed by the range in parentheses. [c]Gf, gallbladder fundus; Gb, gallbladder body; and Gn, gallbladder neck. [d]The locations of three adenomas were not identified because of detachment from the wall.

Table IV. Relationship between gross type and tumor size in 78 pyloric-gland type adenomas of the gallbladder.

Gross type	Tumor size (X), mm			No. of tumors
	X<10 n (%)	10≤X<30 n (%)	X≥30 n (%)	n (%)
Pedunculated type	33	20	4[a]	57 (73%)
Sessile type	20	1	0	21 (27%)
No. of tumors [n (%)]	53 (68%)	21 (27%)	4[a] (5%)	78 (100%)

[a]Two of four adenomas had carcinomatous foci.

Table V. Relative frequencies in accordance with the degree of distribution of positive cells for mucin markers and CD10 in 58 pyloric-gland type adenomas of the gallbladder.

Immuno-histochemical markers	Percentage of reactive cells within total tumor cells (X)				Total tumors
	X=0% n (%)[a]	0%<X<10% n (%)[a]	10%≤X<30% n (%)[a]	X≥30% n (%)[a]	n
MUC5AC	0 (0%)	20 (34%)	16 (28%)	22 (38%)	58
HGM	0 (0%)	15 (26%)	14 (24%)	29 (50%)	58
MUC6	0 (0%)	0 (0%)	0 (0%)	58 (100%)	58
M-GGMC-1	0 (0%)	2 (3%)	2 (3%)	54 (93%)	58
MUC2	16 (28%)	42 (72%)	0 (0%)	0 (0%)	58
CD10	0 (0%)	26 (45%)	12 (21%)	20 (34%)	58

[a]Percentages do not always total 100 because of rounding.

positive reactivity for MUC5AC, MUC6 and MUC2, while expression both in the cytoplasm and on the luminal membrane was positive for HGM and M-GGMC-1. CD10 was regarded as a marker for proper biliary epithelium, when it was expressed at the striated border on the luminal surface of the epithelium. Extracellular luminal mucin reactive for each staining was excluded from this study. When the internal positive or negative controls were not stained as expected, repeated immunostaining was performed for the same tissue blocks. The percentage (X) of reactive cells in relation to the total number of tumor cells was estimated semi-quantitatively, and divided into four categories: X=0% (negative), 0%<X<10%, 10%≤X<30%, and X≥30%.

Analysis of KI-67, p53 and CDX2 immunostaining. P53- and Ki-67-positive cells were defined as reactive cells with staining in the nucleus, regardless of the staining intensity. Cells with intranuclear biotin inclusion were not included among the positive cells. The positive index both for p53 and Ki-67 indicated the ratio of positive cells in relation to the total tumor cells that were counted (at least 500), especially in areas where positive cells were most diffusely distributed. P53 protein overexpression was defined as a focally aggregated or diffuse expression pattern, in contrast to negative or sporadic expression. In the present study, we used CDX2 as an indicator for potential intestinal cell differentiation. Brown staining in the nucleus only was defined as being positive for CDX2 expression, and immnostaining was judged positive when CDX2 was expressed in ≥10% of tumor cells. When <10% of tumor cells were stained, the result was considered to be negative. Moreover, we also performed immunohistochemical staining for CDX2 in 10 normal gallbladders, and in our series of three intestinal type adenomas of the gallbladder, as negative and positive controls, respectively, to compare the positivity with that of the 58 pyloric-gland type adenomas.

Statistical analysis. Statistical analysis was performed using Mann-Whitney's U test or Fisher's exact test to compare the mucin expression profile between normal proper gallbladder epithelium and pyloric-gland type adenomas of the gallbladder. P<0.05 was considered statistically significant.

Results

Clinicopathological features of pyloric-gland type adenoma of the gallbladder. Seventy eight pyloric-gland type adenomas obtained from 59 (0.4%) patients were identified in 13,223 gallbladders removed for inflammation or neoplasms in our institute over the past 23 years. The clinicopathological features of our series of 78 pyloric-gland type adenomas are shown in Table III. Forty-five (76%) of the 59 patients had a solitary tumor, while the remaining 14 (24%) had multiple lesions. The age of the patients ranged from 39 to 86 years, with a mean of 63.8 years. There were 41 (69%) female and 18 (31%) male patients. Thirty-three (69%) of the 48 patients known to have cholelithiasis presented with complications. The most common location of the lesions was the body of the gallbladder, followed by the neck, with 37 and 23 tumors, respectively. The largest diameter of the tumors was 1.0-40.0 mm, with a mean and median of 9.8 and 6.0 mm, respectively. The relationship between the gross type and size of the pyloric-gland type adenomas of the gallbladder is shown in Table IV. Fifty-seven (73%) of 78 lesions were pedunculated, while the remaining 21 (27%) were sessile or
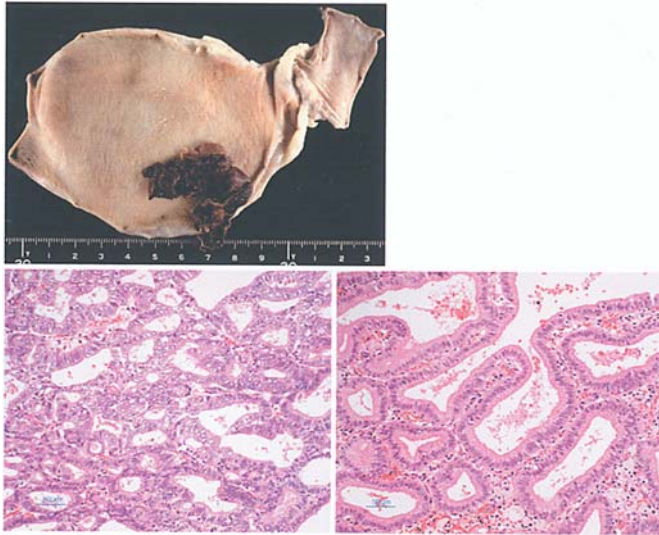
Figure 2. Adenocarcinoma arising from tubular adenoma in a 60-year-old Japanese man: (a) macroscopic findings, (b) H&E section of foci of well-differentiated adenocarcinoma versus (c) component of tubular adenoma of pyloric-gland type (x200).

low elevated lesions. Fifty-one (65%) of the 78 adenomas were small, with a largest diameter <10 mm. On the other hand, four (5%) pedunculated lesions were ≥30 mm in diameter. Two of these four large tumors had focal carcinomatous areas, in which the tumor cells demonstrated a higher degree of atypical cytology (Fig. 2). Briefly, the histopathological criteria used to distinguish carcinoma from adenoma were nuclear size and minor axis, nuclear shape and polarity, chromatin pattern and cytoplasmic features. Areas of carcinomatous transformation arose in the adenomas, either as solitary or multiple foci, where the carcinoma cells were confined to the epithelium, without stromal invasion both in the stalk and in the surrounding mucosa.

Mucin markers and CD10 expression profile based on the immunohistochemical stainings. In relation to the degree of distribution of cells positive for the six immunohistochemical markers, the relative frequency of pyloric-gland type adenoma of the gallbladder is shown in Table V and Fig. 3. MUC5AC, HGM, MUC6, M-GGMC-1 and CD10 were expressed in all 58 pyloric-gland type adenomas of the gallbladder, however,
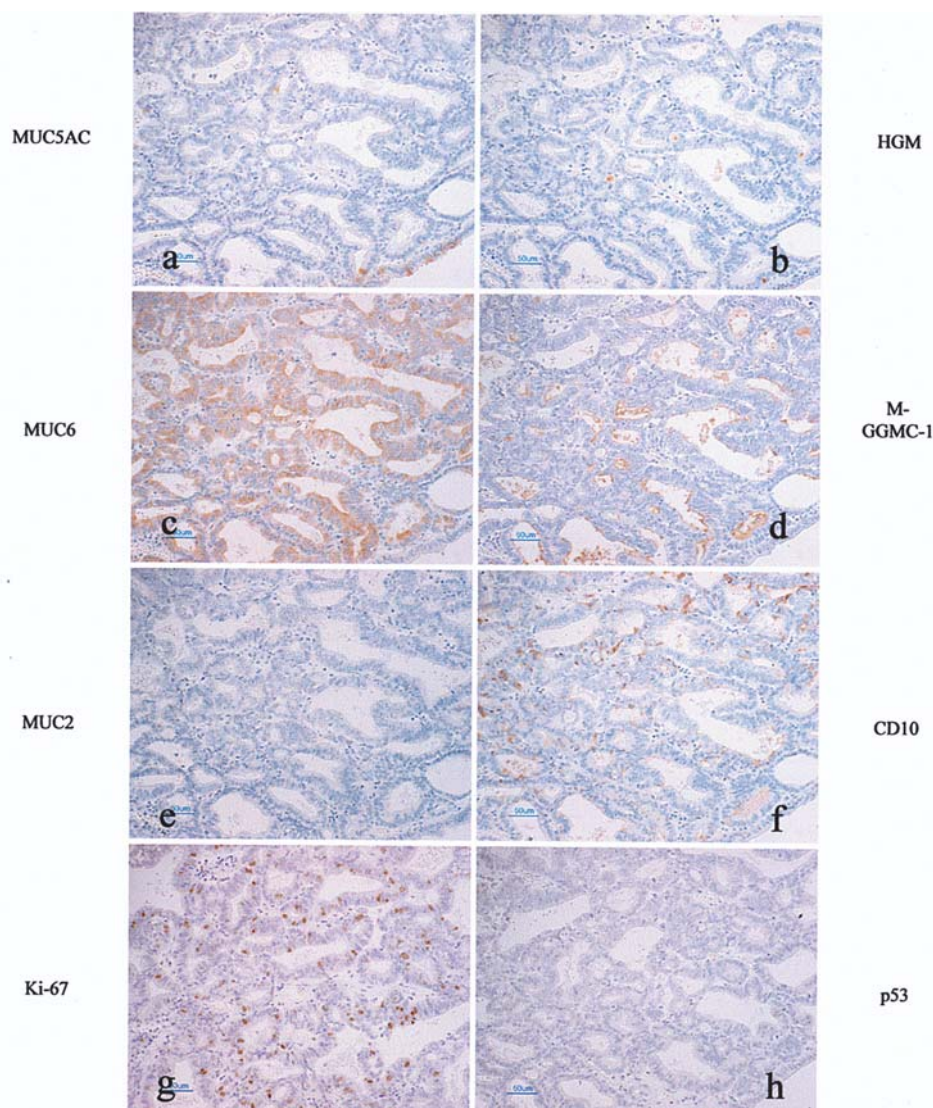

Figure 3. Expression of immunohistochemical markers in pyloric-gland type adenoma of the gallbladder: (a) MUC5AC, (b) HGM, (c) MUC6, (d) M-GGMC-1, (e) MUC2, (f) CD10, (g) Ki-67 and (h) p53 (x200).

Table VI. Comparison of the immunohistochemical expression profile between pyloric-gland type adenomas of the gallbladder and normal gallbladder epithelium.

Immunohistochemical markers		Percentage of reactive cells relative to the total number of tumor cells			P	Total tumors
		0%≤X<10% n (%)[a]	10%≤X<30% n (%)[a]	X≥30% n (%)[a]		n
MUC5AC	NE	0 (0%)	2 (33%)	4 (67%)		6
	AD	20 (34%)	16 (28%)	22 (38%)		58
HGM	NE	1 (17%)	1 (17%)	4 (67%)		6
	AD	15 (26%)	14 (24%)	29 (50%)		58
MUC6	NE	0 (0%)	3 (50%)	3 (50%)	<0.001[b]	6
	AD	0 (0%)	0 (0%)	58 (100%)		58
M-GGMC-1	NE	6 (100%)	0 (0%)	0 (0%)	<0.001[b]	6
	AD	2 (3%)	2 (3%)	54 (93%)		58
MUC2	NE	6 (100%)	0 (0%)	0 (0%)		6
	AD	58 (100%)	0 (0%)	0 (0%)		58
CD10	NE	0 (0%)	0 (0%)	6 (100%)	0.006[c]	6
	AD	26 (45%)	12(21%)	20(34%)		58

NE, normal epithelium of the gallbladder; AD, pyloric-gland type adenomas. [a]Percentages do not always total 100 because of rounding. [b]Stastically compared by Mann-Whitney's U test and Fisher's exact test. [c]Evaluated using Fisher's exact test.

MUC2 expression was not observed in 16 (28%) of the 58 adenomas. Out of the 58 pyloric-gland type adenomas, ≥30% of adenoma cells were positive for MUC5AC in 22 (38%) tumors, HGM in 29 (50%), MUC6 in 58 (100%), M-GGMC-1 in 54 (93%), MUC2 in none (0%), and CD10 in 20 (34%). On the other hand, <10% of adenoma cells were positive for MUC5AC in 20 (34%) tumors, HGM in 15 (26%), MUC6 in 0 (0%), M-GGMC-1 in 2 (3%), MUC2 in 58 (100%), and CD10 in 26 (45%). Dividing the distribution of positive cells into three groups: 0%≤X<10%, 10%≤X<30%, and X≥30%, the relative frequencies of expression for the six immuno-histochemical markers were compared between the 58 pyloric-gland type adenomas and six normal gallbladder epithelia (Table VI). Although there were only a small number of normal gallbladders used for comparison with pyloric-gland type adenomas, as shown in Table VI, statistically significant differences were present between pyloric-gland type adenomas and non-neoplastic gallbladder epithelium in the expression profile of MUC6 (P<0.001), M-GGMC-1 (P<0.001), and CD10 (P=0.006) immunostaining.

Expression of p53, KI-67, and CDX2 in 58 pyloric-gland type adenomas of the gallbladder. P53 overexpression was not found in any of the 58 pyloric-gland type adenomas of the gallbladder, including two adenomas with carcinomatous foci. The Ki-67-positive index was 10.3±5.8% (mean ± SD) in the tumors. The distribution patterns of Ki-67-positive cells in the adenomas were sporadic or very focused without aggregation, however, in the two adenomas with carcinomatous foci, where the tumor cells showed a higher degree of atypical cytology, the Ki-67-positive index was 16.2 and 25.1%, and the positive cells had a characteristic focal aggregation pattern.

CDX2 expression was judged as negative in all the 58 pyloric-gland type adenomas. Before evaluation, we confirmed that CDX2 was not expressed in the epithelium of 10 normal gallbladders (Fig. 4a). In contrast to those immunohisto-chemical results, diffuse expression of CDX2 protein was detected in all of our three intestinal-type adenomas of the gallbladder (Fig. 4b and c).

Discussion

To date, it is well established that MUC2, MUC5AC, and MUC6 are specific immunohistochemical markers for the mucins of intestinal goblet cell, gastric foveolar and pyloric-gland type, respectively (11-15,20-23). Mucin phenotype is generally determined by a combination of immunohisto-chemical tests for the MUC series in various organs. In our institute, both HGM and M-GGMC-1 (or Con A-III) are added to the MUC series when evaluating the mucin expression profile of the gastric phenotype (24-27). In normal human gastric mucosa, HGM (45M1) (28) and α-linked N-acetylglucosamine (GlcNAc) residues (M-GGMC-1 or Con A-III) (29) are expressed in the foveolar epithelium and in the deep glands of the antrum and the mucous neck cells of the corpus, respectively. Both are sometimes detected on the luminal surface coat of the epithelium as well as in the cytoplasm, whereas MUC5AC and MUC6 expression is usually restricted to the cytoplasm (24). This is why we consider that immunostaining for HGM and M-GGMC-1 (or Con A-III), in addition to MUC5AC and MUC6, is useful for evaluating the foveolar and pyloric-gland type mucins, respectively.

Very few reports have described the evaluation of the mucin profile of pyloric-gland type adenomas of the gallbladder by
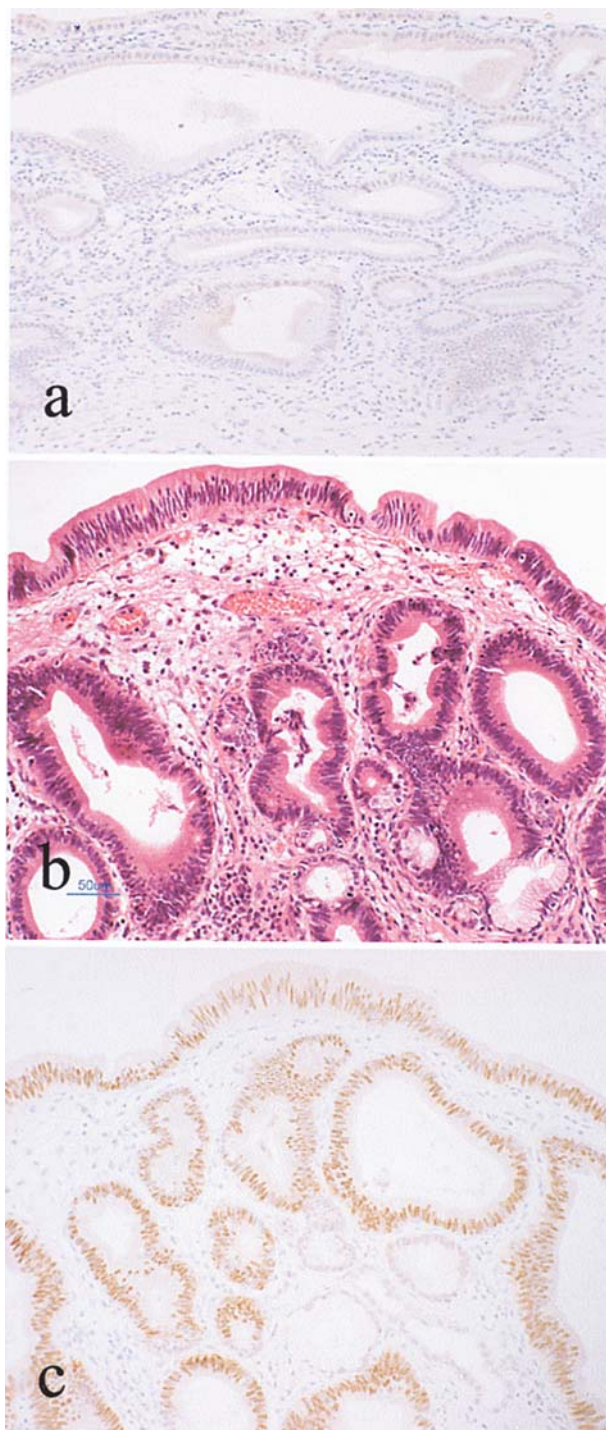
Figure 4. Immunostaining for CDX2. (a) Normal gallbladder epithelium showing no CDX2 expression (x200). (b) H&E section, and (c) diffuse expression of CDX2 protein by immunohistochemical staining of intestinal-type adenoma of the gallbladder (x200).

using the MUC series (8,16). Chang *et al* (8) performed immunohistochemical staining on 31 adenomas of the gall-bladder, comprising 20 pyloric-gland type, nine intestinal type and two biliary type, using MUC2, MUC5AC and MUC6, as well as cytokeratins CK7 and CK20. In their studies, MUC6 expression without MUC2 or CK20 expression was described as being helpful for establishing their criteria for pyloric-gland type adenoma, in which determination of a positive mucin phenotype was set at >10% of positive cells

within the total number of tumor cells (8,16). In our study, MUC6 mucin was detected more often than M-GGMC-1, with ≥30% of adenoma cells positive for MUC6 in 100%, and M-GGMC-1 in 93%, of the total 58 pyloric-gland type adenomas, as shown in Table V. On the other hand, when the percentage of positive mucin phenotype was set at ≥10% of positive cells, all the 58 adenomas were classified into the pyloric-gland phenotype, according to the expression profile of MUC6 and M-GGMC-1 that exhibits highly selective reactivity with class III mucins, which are characteristic for gastric pyloric gland cells, as identified by paradoxical concanavalin A (29). Therefore, we suggest that the percentage of positive cells adopted to define pyloric-gland phenotype should be 10% in pyloric-gland type adenomas of the gallbladder.

The expression profile of CD10 in the biliary epithelium has not yet been well established. CD10, also known as neutral endopeptidase-24.11 or neprilysin (30), is an endopeptidase responsible for reducing the local concentration of biologic modulators by catabolism (31). Although CD10 was originally identified in acute leukemia as common acute lymphoblastic leukemia antigen (CALLA), it has recently been shown to be expressed not only in a variety of hematopoietic neoplasms, but also in non-hematopoietic neoplasms such as carcinoma of the liver, pancreas, kidney, bladder, prostate and uterus, in addition to sarcoma and malignant melanoma (32-35). Moreover, CD10 is also accepted as the most popular brush-border marker of the small intestine and, especially in gastric carcinoma, it is used as intestinal markers as well as MUC2 in determining whether the carcinoma is of the gastric or intestinal phenotype (36,37). As to the biliary tracts, Loke *et al* (38) performed immunostaining using anti-CALLA antibody (CD10) on human hepatic tissue, and observed strong immunostaining for the antigen localized only in the area of the bile canaliculi. Furthermore, by immunoelectron microscopy, the chromogen was confirmed to be present along the surface of the biliary microvilli. Scoazec *et al* (39) immunohistochemically analyzed the expression of polarized plasma membrane proteins of human biliary epithelium, incl-uding neutral endopeptidase (CD10), in the various anatomical compartments of the biliary tree. As a result of their study, they described that the apparent expression level of CD10 was high in the large intrahepatic and extrahepatic bile ducts, as well as in the gallbladder. In our study, CD10 expression at the striated border of the epithelium was also frequent in non-neoplastic gallbladder epithelium, while its expression was significantly reduced in pyloric-gland type adenomas of the gallbladder. Therefore, we consider that the loss of the brush-border-like structure of the normal gallbladder epi-thelium occurs through tumorigenesis towards adenomas of the pyloric-gland phenotype, in which the proper biliary phenotype is diminished. For the first time, we believe that we have demonstrated CD10 expression in pyloric-gland type adenomas of the gallbladder, in comparison with that in normal gallbladder epithelium.

In the present study, we also investigated the expression of CDX2 protein, which is associated with homeobox gene CDX2, in pyloric-gland type adenomas of the gallbladder. Homeobox genes encode nuclear transcription factors involved in the establishment of differentiation patterns during development, and their maintenance in adults (40). Human

CDX2, a member of the caudal-related homeobox gene family, plays a crucial role in the regulation of cell proliferation and differentiation in the gut (17-19). While CDX2 is mostly expressed in the villi or differentiated cell compartment of the intestine (41,42), ectopic expression is observed in intestinal metaplasia of other organs (43-46). In the present study, <10% of cells were positive for CDX2 in all of the 58 pyloric-gland type adenomas, in contrast to its diffuse expression in the three intestinal adenomas of the gallbladder. In addition, normal gallbladder epithelium, as a negative control for immunohistochemistry, in which CD10 expression was frequently seen, was also negative for CDX2. This supports the hypothesis that CD10 expression in the gallbladder epithelium indicates the phenotype of proper biliary epithelium rather than that of the small intestine, in contrast to the situation in determining the phenotype of gastric carcinoma.

In conclusion, pyloric-gland type adenomas of the gallbladder demonstrated high frequency expression of MUC6 and M-GGMC-1, both of which are specific for pyloric glands, whereas MUC2 expression specific for intestinal goblet cells was rarely seen. Moreover, co-expression of MUC5AC and HGM, both of which are specific for gastric foveolar epithelium, and CD10, which is frequently observed in native gallbladder epithelium, was also observed in a considerable proportion of these tumors. This study reveals a differentiation of pyloric-gland type adenomas of the gallbladder towards pyloric glands in terms of immunohisto-chemistry as well as morphology, and that they also have the cytological phenotype of gastric foveolar and native gallbladder epithelium. Future research should determine whether this heterogeneity in the mucin expression profile of pyloric-gland type adenomas of the gallbladder is caused by chronological differences in the tumorigenesis or variation in epithelial origin (e.g. metaplastic pyloric type epithelium or proper biliary epithelium) or by the biological nature of this tumor.

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