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Mucin-histochemical and immunohistochemical profiles of epithelial cells of several types of hepatic cysts

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Summary. Epithelial cells of several types of hepatic cysts were examined by mucin histochemistry and immunohistochemically. There were some differences in mucus and antigenic expression among the hepatic cysts examined. Epithelial cells of non-parasitic simple cysts and adult-type polycystic liver showed similar mucin-histochemical and immunohistochemical features, and were characterized by little mucin and weak immunoreactivities to several antibodies examined. Epithelial cells of hepatic hilar cysts were characterized by much mucin and moderate immunoreactivities to carbohydrate antigen 19-9 (CA 19-9), carcinoembryonic antigen (CEA) and epithelial membrane antigen (EMA). Epithelial cells of ciliated hepatic foregut cysts were characterized by much mucin and immunoreactivities to actin and tubulin which were positive in cilia. Epithelial cells of biliary cystadenoma were characterized by much mucin and moderate to strong immunoreactivities to cytokeratins CAM5.2 and AE1 and 3 as well as to CA 19-9, CEA, EMA and DU-PAN-2. Epithelial cells of biliary cystadenocarcinoma were characterized by much mucin and moderate to strong immunoreactivities to cytokeratins CAM5.2 and AE1 and 3 as well as to CA 19-9, CEA, EMA and DU-PAN-2. These differences in epithelial mucus and antigenic expression among several types of hepatic cysts may reflect differences in their origin and biological characteristics. These differences may be helpful in the differential diagnosis of hepatic cysts in small biopsy specimens.

Key words: Hepatic cysts – Mucin histochemistry – Immunohistochemistry – Intrahepatic bile ducts

Introduction

Several types of cysts occur in the liver. They include non-parasitic simple cysts, parasitic (echinococcal hydatid) cysts, multiple cysts of adult-type polycystic disease (Melnick 1954), hepatic hilar cysts (Nakanuma et al. 1984; Terada and Nakanuma 1990), ciliated hepatic foregut cysts (Wheeler and Edmondson 1984; Terada et al. 1990), biliary cystadenoma (Ishak et al. 1977) and biliary cystadenocarcinoma (Ishak et al. 1977). These hepatic cysts are lined by biliary-type epithelia, and are different from one another in their morphology, origin and biological behaviour.

Recent advances in mucin histochemistry and immunohistochemistry have revealed that many epithelial cell types express different kinds of mucin and antigens. In the biliary tract, several types of mucin and antigens are known to be expressed in biliary epithelial cells. However, there have been no reports that describe mucin-histochemical and immunohistochemical findings in hepatic cysts.

In the present study, we examined several types of hepatic cysts in order to see whether there are any differences in mucus and antigenic expression in the cystic epithelial cells. Some differences in their expression among the liver cysts examined was found.

Materials and methods

We retrieved hepatic cysts from our surgical and autopsy files during the period between 1980 and 1990. They included eight cases of non-parasitic simple cysts (two surgically resected cases and six autopsy cases), seven autopsy cases of adult-type polycystic disease, five autopsy cases of hepatic hilar cysts, four surgically resected cases of ciliated hepatic foregut cysts, four surgically resected cases of biliary cystadenoma and three surgically resected cases of biliary cystadenocarcinoma. Of the seven cases of adult-type polycystic disease, six were associated with polycystic kidneys. Of the four cases of biliary cystadenoma, two contained focal malignant areas. The morphological features of three of the four ciliated hepatic foregut cysts have been reported previously (Terada et al. 1990). In autopsy cases, several tissue specimens including cysts and marginal liver tissue were obtained. In surgically resected cases, one or several specimens including cysts and marginal liver tissue were available. The liver tissue specimens thus obtained were fixed in 10% formalin and embedded in paraffin. Between 20 and

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Table 1. Immunohistochemical antibodies used in this study

Antibodies	Source	Dilution
Primary antibodies		
Keratin (P)	Rabbit (Dako, Sanata Barbara, Calif.)	1/400
Cytokeratin (M) CAM 5.2	Mouse (Becton-Dickson, Mountain View, Calif.)	1/50
Cytokeratin (M) AE1 and 3	Mouse (Hydritech, San Diego, Calif.)	1/50
DU-PAN-2 (M)	Mouse (Kyowa Medics, Japan)	1/500
CA 19-9 (M)	Mouse (Midori Juji, Japan)	Prediluted
CEA (P)	Rabbit (Dako)	1/200
EMA (M)	Mouse (Dako)	1/100
Secretory (P) component	Rabbit (Dako)	1/300
IgA (P)	Rabbit (Dako)	1/300
IgM (P)	Rabbit (Dako)	1/300
Actin[a] (M)	Mouse (Amersham, UK)	1/500
Tubulin[a] (P)	Rabbit (BioMarkor, Israel)	1/10
Desmin[a] (M)	Mouse (Dako)	1/30
Secondary biotinylated antibodies		
Antirabbit immunoglobulins	Swine (Dako)	1/500
Antimouse immunoglobulins	Rabbit (Dako)	1/300
Antimouse IgM	Goat (Cappel, Malvem, Pa.)	1/100
Antimouse IgG	Horse (Vector Laboratories, Calif.)	1/200

P, Polyclonal antibody; M, monoclonal antibody; EMA, epithelial membrane antigen; CEA, carcinoembryonic antigen; CA 19-9, carbohydrate antigen 19-9.
[a] Pre-digested by 0.1% trypsin solution

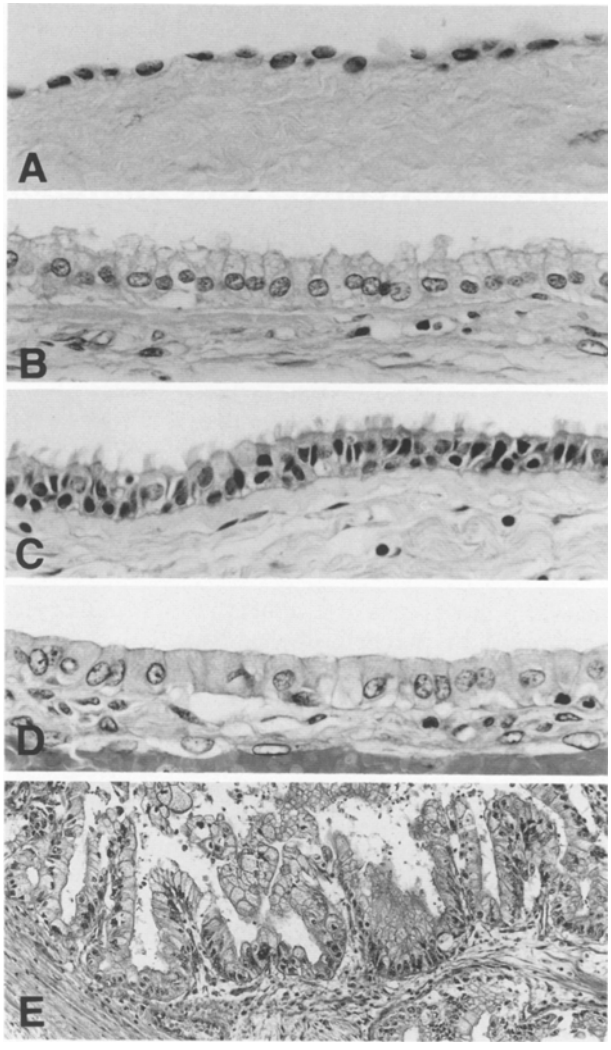

Fig. 1. Epithelial cells of non-parasitic simple cyst (**A**), hepatic hilar cyst (**B**), ciliated hepatic foregut cyst (**C**), biliary cystadenoma (**D**), and biliary cystadenocarcinoma (**E**). Haematoxylin and eosin, ×200

30 sections approximately 5 μm thick were cut from each paraffin block, and stained with haematoxylin and eoxin, elastic van Gieson, silver impregnation, periodic acid-Schiff (PAS), and Azan-Mallory. Combined alcian blue at pH 2.5 and PAS stain and combined high iron diamine and alcian blue at pH 2.5 stain were also employed for mucin histochemistry.

Immunohistochemistry was done using the avidin-biotin-peroxidase method after Hsu et al. (1981). The antibodies used and their source and dilution are shown in Table 1. Briefly, the paraffin sections were deparaffinized and immersed for 20 min in methanolic hydrogen peroxide to block endogenous peroxidase activity. Then, the sections were treated with normal sera for 20 min. The sections were washed by phosphate-buffered saline (PBS), and primary antibodies were applied to the sections for 60 min. After washing with PBS, the sections were treated with secondary biotinylated antibodies for 30 min. Then, the sections were washed with PBS, and treated with the avidin-biotin-peroxidase complex (Vectastain ABC Kit, Vector Laboratories, Burlingame, Calif., USA) for 30 min. The sections were developed with 3-3′diaminobenzidine tetrahydrochloride solution with hydrogen peroxide, and slightly counterstained with haematoxylin. Intrahepatic bile ducts and peribiliary glands (Terada et al. 1987) of marginal liver tissue were used as intrinsic controls.

Results

Grossly, non-parasitic simple cysts were unilocular and contained serous fluid. Adult-type polycystic livers harboured multiple unilocular cysts containing serous fluid. Hepatic hilar cysts were unilocular, were located around intrahepatic bile ducts in the hepatic hilus, and contained seromucous fluid. Ciliated hepatic foregut cysts were unilocular, and contained mucous fluid. Biliary cystadenoma was multilocular and contained mucous fluid. Biliary cystadenocarcinoma was also multilocular and contained mucous fluid.

Histologically, non-parasitic simple cysts consisted of a single layer of cuboidal or flattened epithelium (Fig. 1 A). Liver cysts of adult-type polycystic livers also consisted of a single layer of cuboidal or flattened epithelium. There were many microhamartomas (von Meyenburg complexes) in the polycystic livers. Hepatic hilar cysts consisted of a single layer of cuboidal or culumnar epithelia (Fig. 1 B). Ciliated hepatic foregut cysts con-

Table 2. Mucin-histochemical and immunohistochemical findings of epithelial cells of hepatic cysts

Case no.	Age/sex	AB/PAS	HID/AB	Keratin	CAM 5.2	AE1+3	DU-PAN-2	CA19-9	CEA	EMA	SC	IgA	IgM	Actin	Tubulin
Non-parasitic simple cysts															
1 (A)	54/F	+	−	+	+	+	−	+	−	+	−	−	−	−	−
2 (A)	75/M	+	+	+	2+	2+	−	+	+	+	−	−	−	−	−
3 (S)	64/M	+	−	+	+	2+	−	+	+	+	−	−	−	−	−
4 (S)	62/F	−	−	+	+	2+	+	+	+	+	+	−	−	−	−
5 (A)	49/M	−	−	+	+	+	−	+	−	+	+	−	−	−	−
6 (A)	47/F	−	−	+	+	+	−	+	+	+	−	−	−	−	−
7 (A)	54/F	+	−	−	+	+	−	+	−	+	−	−	−	−	−
8 (A)	64/M	−	−	−	+	+	−	+	−	+	−	−	−	−	−
Adult-type polycystic livers															
1 (A)	75/F	+	−	+	+	+	+	+	+	+	+	−	−	−	−
2 (A)	60/F	+	−	+	2+	2+	−	+	+	+	+	−	−	−	−
3 (A)	67/M	+	−	+	2+	2+	+	+	+	+	+	−	−	−	−
4 (A)	72/M	+	+	+	+	+	−	+	+	+	+	−	−	−	−
5 (A)	60/M	+	−	+	2+	+	+	+	+	+	+	−	−	−	−
6 (A)	63/F	+	−	+	+	+	+	+	+	+	+	−	−	−	−
7 (A)	77/M	−	−	+	+	+	−	+	+	+	+	−	−	−	−
Hepatic hilar cysts															
1 (A)	50/F	3+	2+	+	2+	+	+	2+	2+	2+	+	−	−	−	−
2 (A)	66/M	2+	+	+	2+	+	−	+	2+	2+	−	−	−	−	−
3 (A)	70/M	3+	2+	+	+	+	+	2+	+	2+	+	−	−	−	−
4 (A)	78/M	3+	3+	+	2+	+	+	+	2+	2+	2+	−	−	−	−
5 (A)	54/M	2+	+	+	2+	2+	−	2+	2+	−	+	−	−	−	−
Ciliated hepatic foregut cysts															
1 (S)	59/F	3+	2+	+	+	+	+	+	2+	+	+	−	−	3+	3+
2 (S)	69/M	2+	2+	+	+	+	+	+	2+	+	−	−	−	3+	3+
3 (S)	41/M	3+	3+	+	+	+	+	+	2+	+	−	−	−	3+	3+
4 (S)	72/F	2+	2+	+	+	+	+	+	+	+	−	−	−	−	−
Biliary cystadenoma															
1 (S)	52/F	2+	2+	+	3+	3+	2+	3+	2+	3+	+	−	−	−	−
2 (S)	69/F	3+	3+	+	3+	+	+	+	+	+	+	−	−	−	−
Biliary cystadenoma with malignant area															
1 (S)	53/F														
Benign area		2+	3+	+	+	2+	2+	2+	2+	2+	+	−	−	−	−
Malignant area		2+	2+	+	2+	2+	2+	2+	2+	2+	+	−	−	−	−
2 (S)	53/F														
Benign area		3+	3+	+	+	+	+	+	+	+	+	−	−	−	−
Malignant area		3+	3+	+	3+	2+	2+	+	2+	3+	+	−	−	−	−
Biliary cystadenocarcinoma															
1 (S)	57/M	+	+	+	3+	3+	3+	2+	+	3+	−	−	−	−	−
2 (S)	73/M	3+	3+	+	3+	2+	+	+	2+	2+	−	−	−	−	−
3 (S)	67/M	3+	3+	+	2+	2+	+	+	2+	2+	−	−	−	−	−
Intrahepatic bile ducts															
		+	+	+	2+	2+	+	2+	2+	+	+	−	−	−	−
Intrahepatic peribiliary glands															
		2+	2+	+	2+	2+	+	2+	2+	+	+	−	−	−	−

A, Autopsy case; S, surgically resected case; AB/PAS, combined alcian blue at pH 2.5 and periodic acid-Schiff; HID/AD, combined high iron diamine and alcian blue at pH 2.5; CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; EMA, epithelial membrane antigen; SC, secretory component; 3+, strong reactivity; 2+, moderate reactivity; +, weak reactivity; −, faint or negative reactivity

sisted of pseudostratified ciliated epithelium (Fig. 1C), smooth muscle bands and the outermost fibrous bands. Biliary cystadenoma with or without malignant areas consisted of a single layer of columnar epithelia (Fig. 1D), and the epithelial cells often showed papillary infolding and invagination. Biliary cystadenocarcinoma consisted of a single or multilayered columnar epithelial cells with cytological atypia (Fig. 1E), and the epithelial

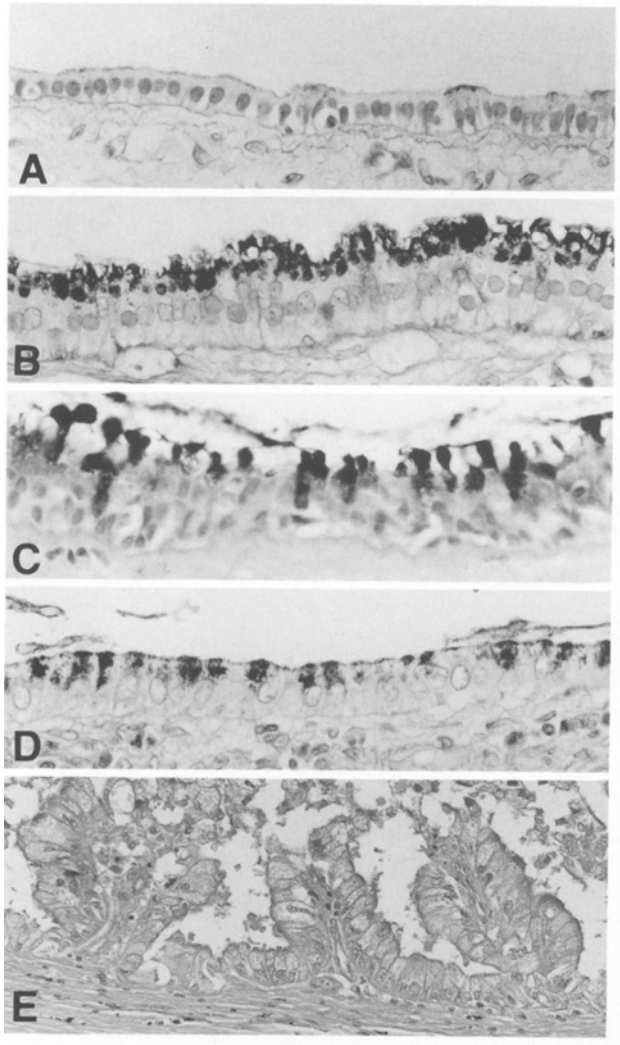

Fig. 2. Mucin-histochemistry of non-parasitic simple cyst (**A**), hepatic hilar cyst (**B**), ciliated hepatic foregut cyst (**C**), biliary cystadenoma (**D**) and biliary cystadenocarcinoma (**E**). Combined alcian blue at pH 2.5 and periodic acid-Schiff stain, ×200

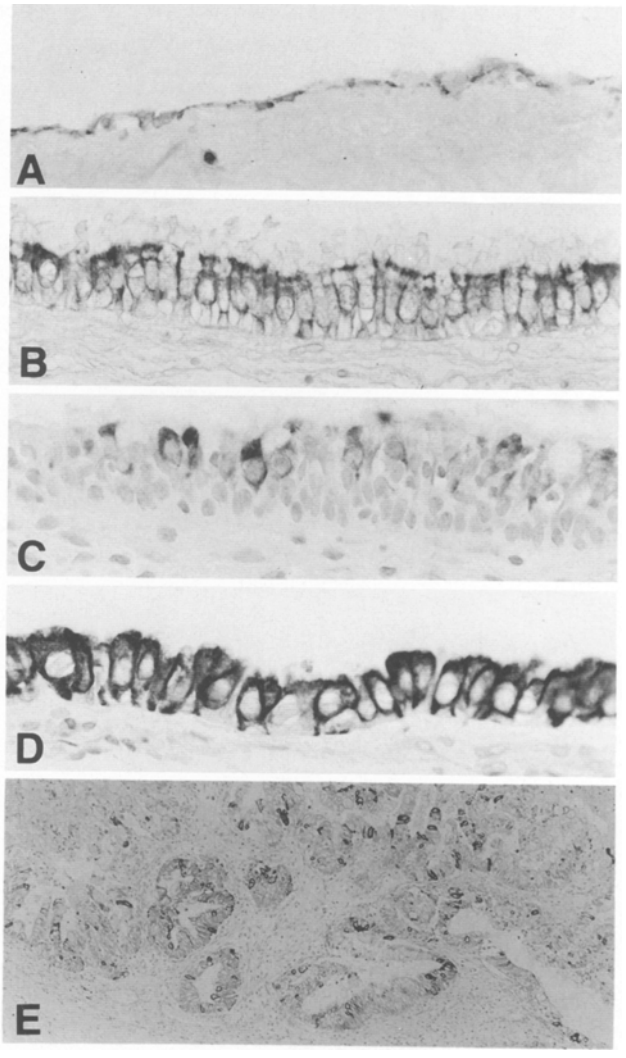

Fig. 3. Cytokeratin CAM 5.2 immunoreactivity in non-parasitic simple cyst (**A**), hepatic hilar cyst (**B**), ciliated hepatic foregut cyst (**C**), biliary cystadenoma (**D**) and biliary cystadenocarcinoma (**E**). **A–D**, ×200; **E**, ×80

cells often showed papillary proliferations and invasion into the underlying stroma (Fig. 1E). Among the cysts, non-dilated adenocarcinoma elements were recognized.

The mucin-histochemical findings are shown in Table 2. Epithelial cells of non-parasitic simple cysts and adult-type polycystic livers harboured a little mucin only at the luminal borders (Fig. 2A). Epithelial cells of microhamartomas also showed similar mucin quantity and localization to those of the cysts. Epithelial cells of hepatic hilar cysts contained much neutral mucin, sialomucin and sulphomucin in the supranuclear cytoplasm (Fig. 2B). Epithelial cells of ciliated hepatic foregut cysts contained much neutral mucin, sialomucin and sulphomucin in the supranuclear cytoplasm (Fig. 2C). Epithelial cells of biliary cystadenoma and benign epithelial cells of cystadenoma with malignant areas contained much neutral mucin, sialomucin and sulphomucin in the supranuclear cytoplasm (Fig. 2D). Epithelial cells of biliary cystadenocarcinoma and malignant epithelial cells of cystadenoma with malignant areas also harboured much neutral mucin, sialomucin and sulphomucin in the supranuclear cytoplasm (Fig. 2E).

The immunohistochemical findings are shown in Table 2. Epithelial cells of non-parasitic simple cysts and adult-type polycystic livers showed similar immunohistochemical findings. They showed moderate immunoreactivities to cytokeratin CAM 5.2 (Fig. 3A) and cytokeratin AE1 and 3, weak immunoreactivities to keratin, DU-PAN-2, carbohydrate antigen 19-9 (CA 19-9) (Fig. 4A), carcinoembryonic antigen (CEA), epithelial membrane antigen (EMA) (Fig. 5A) and secretory component (SC), and negative immunoreactivities to IgA, IgM, actin and tubulin. Epithelial cells of microhamartomas also showed nearly the same immunohistochemical findings as those of the cysts.

Epithelial cells of hepatic hilar cysts showed moderate immunoreactivities to cytokeratin CAM 5.2 (Fig. 3B), cytokeratin AE1 and 3, CA 19-9 (Fig. 4B), and EMA

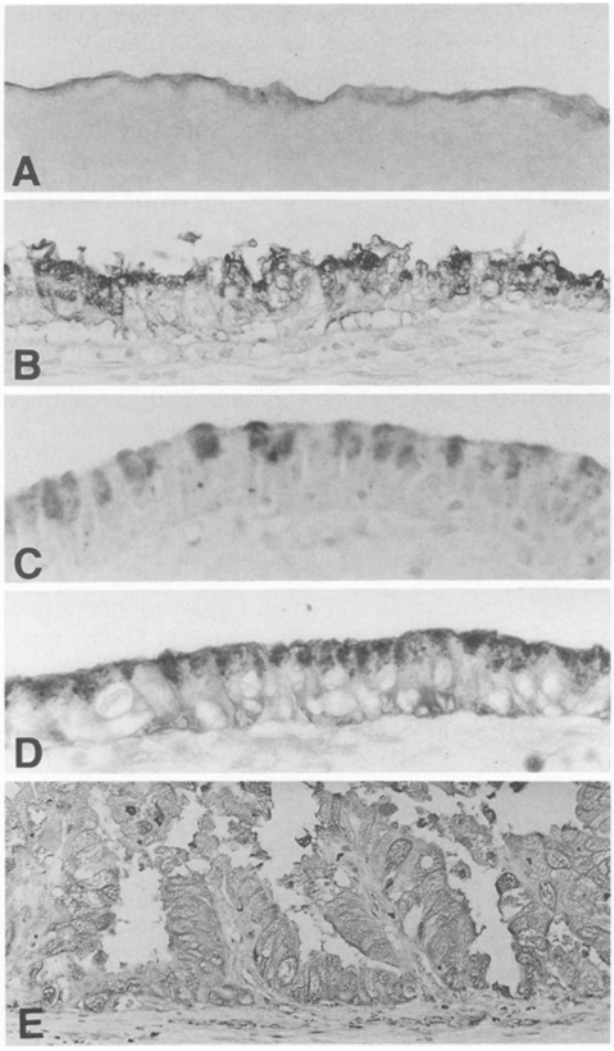

Fig. 4. Carbohydrate antigen 19-9 immunoreactivity in non-parasitic simple cyst (**A**), hepatic hilar cyst (**B**), ciliated hepatic foregut cyst (**C**), biliary cystadenoma (**D**) and biliary cystadenocarcinoma (**E**). ×200

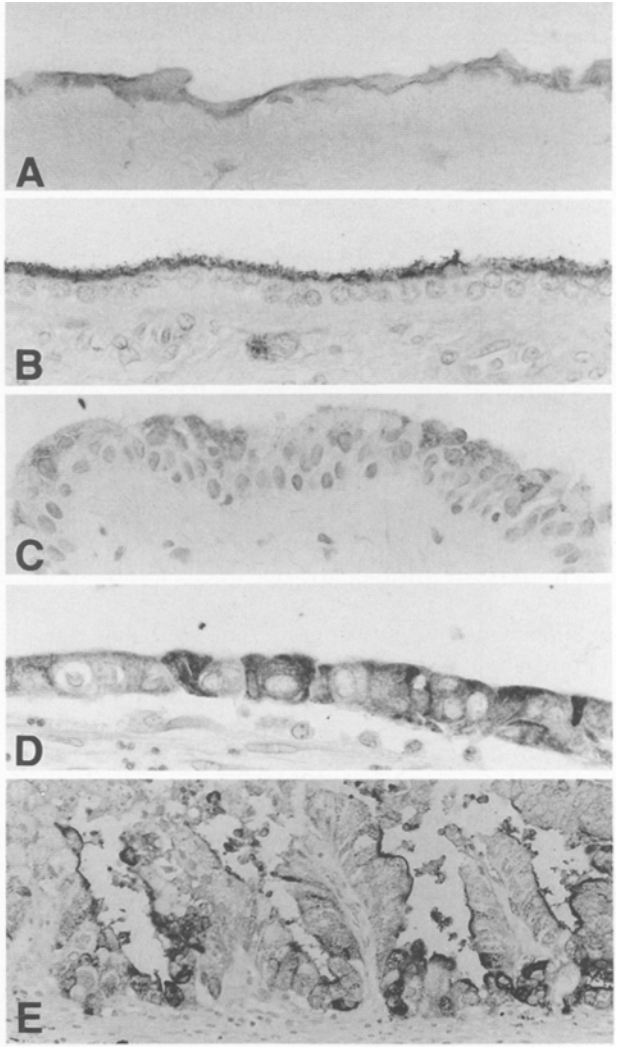

Fig. 5. Epithelial membrane antigen immunoreactivity in non-parasitic simple cyst (**A**), hepatic hilar cyst (**B**), ciliated hepatic foregut cyst (**C**), biliary cystadenoma (**D**) and biliary cystadenocarcinoma (**E**). ×200

(Fig. 5B), weak immunoreactivities to keratin, DU-PAN-2 and SC, and negative immunoreactivities to IgA, IgM, actin and tubulin.

Epithelial cells of ciliated hepatic foregut cysts showed moderate immunoreactivities to CEA, and weak immunoreactivities to keratin, cytokeratin CAM 5.2 (Fig. 3C), cytokeratin AE1 and 3, DU-PAN-2, CA 19-9 (Fig. 4C), EMA (Fig. 5C) and SC, and negative immunoreactivities to IgA and IgM. They also showed strong immunoreactivities to tubulin and actin in three cases. The tubulin and actin immunoreactivities were found in the luminal cilia. Desmin- and actin-positive smooth muscle bands were found in all ciliated hepatic foregut cysts, but were not found in other types of hepatic cysts.

Epithelial cells of biliary cystadenoma and benign epithelial cells of biliary cystadenoma with malignant areas showed moderate to strong immunoreactivities to cytokeratin CAM 5.2 (Fig. 3D), cytokeratin AE1 and 3, CA 19-9 (Fig. 4D), EMA (Fig. 5D), CEA and DU-PAN-2, weak immunoreactivities to keratin and SC, and negative immunoreactivities to IgA, IgM, actin and tubulin.

Epithelial cells of biliary cystadenocarcinoma and malignant epithelial cells of biliary cystadenoma with malignant areas showed moderate to strong immunoreactivities to cytokeratin CAM 5.2 (Fig. 3E) cytokeratin AE1 and 3, CA-19-9 (Fig. 4E), CEA, DU-PAN-2 and EMA (Fig. 5E), weak immunoreactivities to keratin, and no immunoreactivities to SC, IgA, IgM, actin and tubulin.

Intrahepatic bile ducts showed a little neutral mucin, sialomucin and sulphomucin only at the luminal borders (Table 2). Intrahepatic peribiliary glands (Terada et al. 1987), however, harboured more neutral mucin, sialomucin and sulphomucin in the supranuclear cytoplasm (Table 2). Immunohistochemically, intrahepatic bile ducts and peribiliary glands showed moderate immunoreactivities to cytokeratin CAM 5.2, cytokeratin AE1 and

3, CA 19-9 and CEA, weak immunoreactivities to keratin, DU-PAN-2, EMA and SC, and negative immunoreactivities to IgA, IgM, actin and tubulin (Table 2).

Discussion

Most hepatic cysts are lined by biliary-type epithelial cells. We examined mucin and several antigens that are known to be expressed on biliary epithelial cells and found some differences in mucous and antigenic expression of epithelial cells among the several types of hepatic cysts. Epithelial cells of non-parasitic simple cysts and adult-type polycystic livers showed similar mucin-histochemical and immunohistochemical features, suggesting that both of them share common pathogenesis. Their histochemical and immunohistochemical findings were similar to those of intrahepatic bile ducts, although their intensity was weaker in these cysts than in normal biliary epithelium. This finding suggests that epithelial cells of both non-parasitic simple cysts and adult-type polycystic disease are derived from biliary epithelium, and also supports the theory that these cysts are developmental anomalies and arise from the misplaced or detached biliary anlage (Longmire et al. 1971). The finding that epithelial cells of microhamartomas showed similar mucin-histochemical and immunohistochemical findings to those of cysts of adult-type polycystic livers suggests that multiple cysts of the polycystic liver arise from cystic dilatation of microhamartomas.

Epithelial cells of hepatic hilar cysts were characterized by much mucin and moderate immunoreactivities to CA 19-9, CEA and EMA. Normal intrahepatic peribiliary glands also showed similar mucous and antigenic expression, suggesting that hepatic hilar cysts were derived from the cystic dilatation of the peribiliary glands.

Epithelial cells of ciliated hepatic foregut cysts were characterized by much mucin and immunoreactivity to actin and tubulin that were positive in cilia. This finding and the presence of smooth muscle, which was positive for desmin and actin, suggest that this type of hepatic cyst is of developmental origin and arises from embryonic foregut that differentiated into bronchial structures.

Epithelial cells of biliary cystadenoma and benign epithelial cells of cystadenoma with malignant areas were characterized by much mucin and moderate to strong immunoreactivities to cytokeratins CAM 5.2 and AE1 and 3 as well as to DU-PAN-2, CA 19-9, CEA and EMA. The mucus and antigenic expression was stronger in cystadenoma than in normal bile ducts, implying that cystadenoma differed in mucus and antigenic expression from bile ducts. Biliary cystadenoma has been considered as a forerunner of cystadenocarcinoma and our two cases of cystadenoma contained malignant foci. There were only slight differences in the mucus and antigenic expression between cystadenoma and cystadenocarcinoma, suggesting that biliary cystadenoma is a preneoplastic or already neoplastic lesion of cystadenocarcinoma. The rather strong immunoreactivities to CA 19-9, CEA and DU-PAN-2, which are cancer-associated antigens, also support this suggestion.

Epithelial cells of biliary cystadenocarcinoma and malignant epithelial cells of cystadenomas with malignant areas were characterized by much mucin and moderate to strong immunoreactivities to CAM 5.2, AE1 and 3, DU-PAN-2, CA-19-9, CEA and EMA. Although there were only slight differences in these tumour-associated antigens between cystadenoma and cystadenocarcinoma, the rather strong expression of these tumour-associated antigens on malignant cells may imply that antigenic expression alters in carcinogenetic processes of bile ducts.

Finally, it is very plausible that differences in epithelial mucous and antigenic expression among several types of hepatic cysts reflect differences in their origin and biological characteristics. Further, these differences may be helpful in the differential diagnosis of hepatic cysts in small biopsy specimens.

References

Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabelled antibody (PAP) procedures. J Histochem Cytochem 29:577–580

Ishak KG, Willis GW, Cummins SD, Bullock AA (1977) Biliary cystadenoma and cystadenocarcinoma: report of 14 cases and review of the literature. Cancer 38:322–338

Longmire WP, Mandiola SA, Gordon HE (1971) Congenital cystic disease of the liver and biliary system. Ann Surg 174:711–725

Melnick PJ (1954) Polycystic liver: analysis of 70 cases. Arch Pathol Lab Med 59:162–172

Nakanuma Y, Kurumaya H, Ohta G (1984) Multiple cysts in the hepatic hilum and their pathogenesis. A suggestion of periductal gland origin. Virchows Arch [A] 404:341–350

Terada T, Nakanuma Y (1990) Pathological observations of intrahepatic peribiliary glands in 1,000 consecutive autopsy livers. III. Survey of necroinflammation and cystic dilatation. Hepatology 12:1229–1233

Terada T, Nakanuma Y, Ohta G (1987) Glandular elements around the intrahepatic bile ducts in man: their morphology and distribution in normal livers. Liver 7:1–8 19

Terada T, Nakanuma Y, Kono K et al. (1990) Ciliated hepatic foregut cyst: a mucous histochemical, immunohistochemical, and ultrastructural study in three cases in comparison with normal bronchi and intrahepatic bile ducts. Am J Surg Pathol 14:356–363

Wheeler DA, Edmondson HA (1984) Ciliated hepatic foregut cyst. Am J Surg Pathol 8:467–470