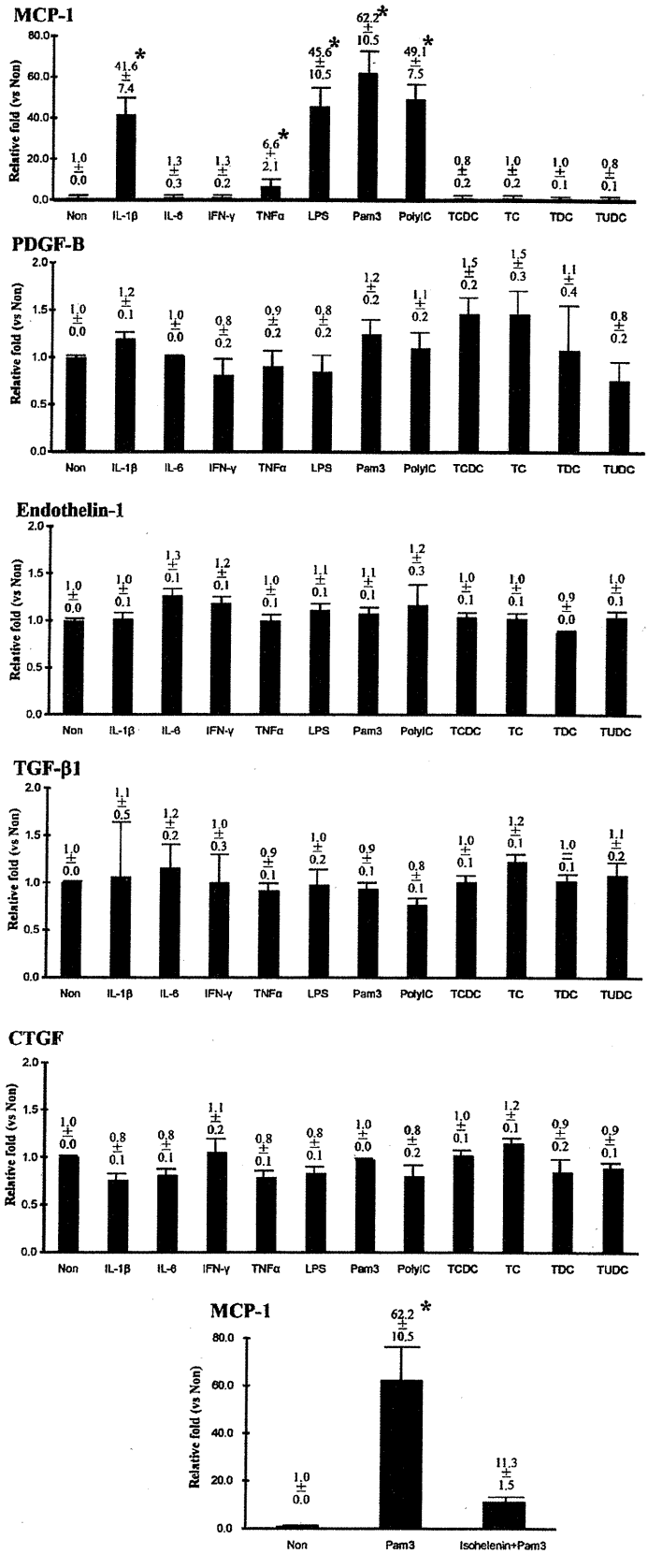

Figure 2 Quantitative PCR analysis for mRNA of fibrogenic cytokines (monocyte chemoattractant protein-1 (MCP-1), platelet-derived growth factor (PDGF)-B, connective tissue growth factor (CTGF), transforming growth factor-β (TGF-β)1, and endothelin-1). MCP-1 expression alone was upregulated by stimulation with Toll-like receptor (TLR) ligands (lipopolysaccharide (LPS), Pam3CSK4, and poly(I:C)) and cytokines (interleukin-1β (IL-1β) and tumour necrosis factor-alpha (TNF-α)). No bile acids (taurochenodeoxycholic acid (TCDC), taurocholic acid (TC), taurodeoxycholic acid (TDC) or tauroursodeoxycholic acid (TUDC)) significantly upregulated MCP-1 mRNA expression. Other fibrogenic cytokines were not affected by any stimulants. A lower figure denotes that Pam3CSK4-induced upregulation of MCP-1 mRNA expression was inhibited by pretreatment with an NF-κB inhibitor, isohelenin. Bars indicate the mean±SEM. *<0.05. INF-γ, interferon-γ.

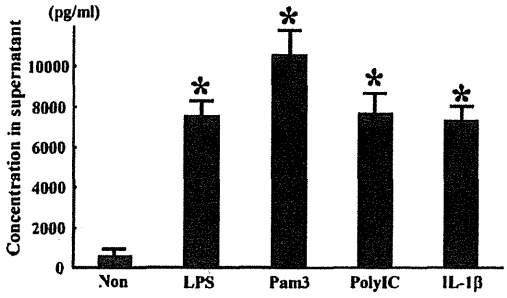

Figure 3 Measurement of monocyte chemoattractant protein-1 (MCP-1) protein by ELISA. After stimulation with lipopolysaccharide (LPS), Pam3CSK4 (Pam3), poly(I:C), and IL-1β for 24 h, the concentration of MCP-1 protein in cultured supernatants of human biliary epithelial cells was significantly increased. Bars indicate the mean±SEM. *<0.05.

MCP-1, but the intensity was similar to or less than that in bile ductules. In contrast, MCP-1-positive bile ductules were not found in CHF. Moreover, the expression of αSMA was limited in portal tracts, and αSMA-positive HSCs were not found in CHF.

Characterisation of CCR2-expressing cells

Fluorescence double immunostaining revealed that cells double positive for CD68 and CCR2 were scattered mostly in interface areas of CVH and PBC, but cells double positive for αSMA and CCR2 were not found in any area (figure 5).

DISCUSSION

Chronic liver injury is generally characterised by inflammation and subsequent fibrosis, and, regardless of the aetiology, a cytokine-rich environment caused by inflammation and infection is closely associated with hepatic fibrosis. HSC is considered the most important effecter cell associated with fibrogenesis in hepatic parenchyma including the interface between portal tracts and periportal hepatocytes, and the fibrous enlargement of portal tracts and fibrous extension from portal areas are closely associated with activated HSCs and their transformed version, myofibroblasts. Within portal tracts, in contrast, fibroblasts and fibrocytes are also important to the histogenesis of portal fibrosis. Several cytokines such as PDGF-B, CTGF, TGF-β, endothelin-1 and MCP-1, are reported as fibrogenetic factors in liver.[5] These cytokines could chemoattract HSCs or directly activate the production of collagen in myofibroblasts.

We have previously reported that human BECs possess receptors for inflammatory cytokines (IL-1β, IL-6, Interferon-γ and TNF-α) and an innate immune system consisting of TLRs.[6 8 9] Therefore, in this study, we examined whether BECs contribute to hepatic fibrosis using cultured human BECs. Consequently, although mRNAs of all fibrogenic cytokines were detected in

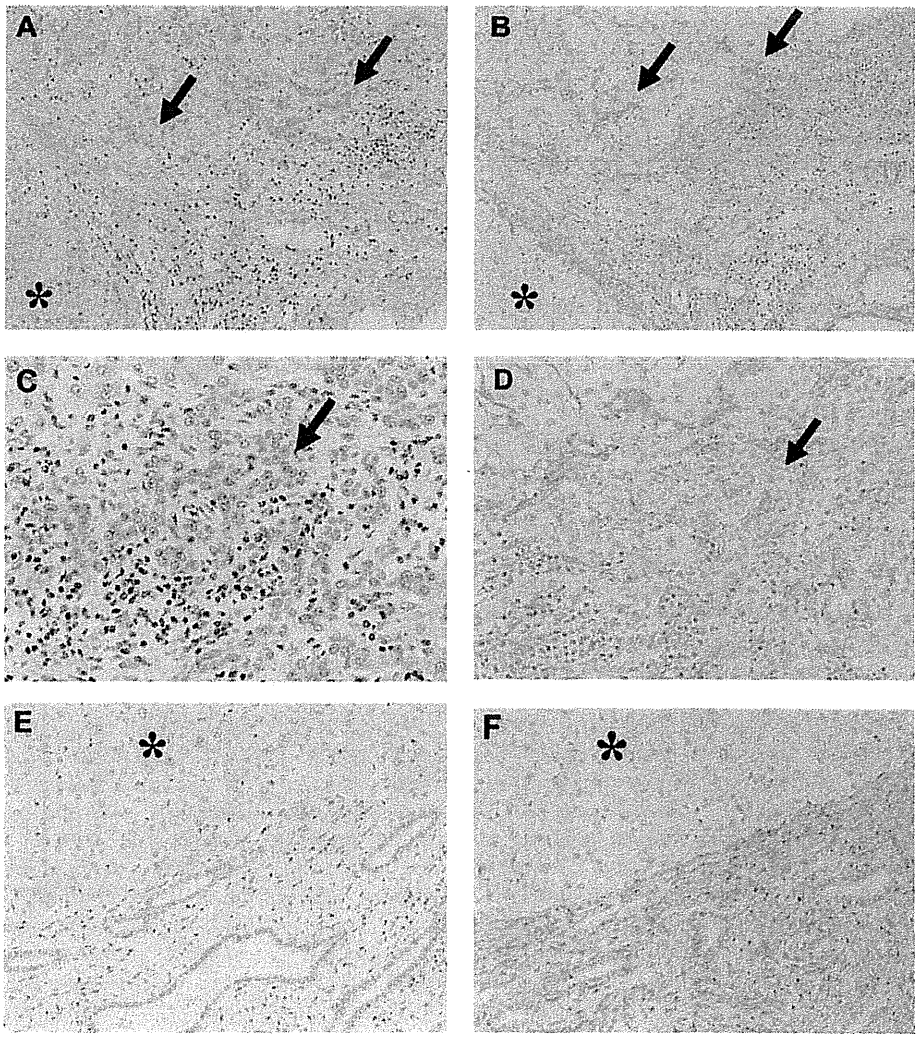

Figure 4 Immunohistochemistry for monocyte chemoattractant protein-1 (MCP-1) (A, C, E) and α-smooth muscle actin (αSMA) (B, D, F). (A, B) Hepatitis C virus-related chronic viral hepatitis. Proliferating bile ductules at the interface of periportal areas express MCP-1 (A, arrows) and αSMA-positive cells showing hepatic stellate cell (HSC) morphology are found in the same area (B, arrows). In contrast, αSMA-positive HSCs are not found in periportal parenchyma lacking bile ductules (asterisks). (C, D) Primary biliary cirrhosis. MCP-1-positive proliferating bile ductules and αSMA-positive cells are intermingled (arrows). (E, F) Congenital hepatic fibrosis. No biliary bile ducts or ductules express MCP-1, and no αSMA-positive HSCs are found in periportal areas (asterisks). The expression of αSMA is limited in portal tracts (lower right).

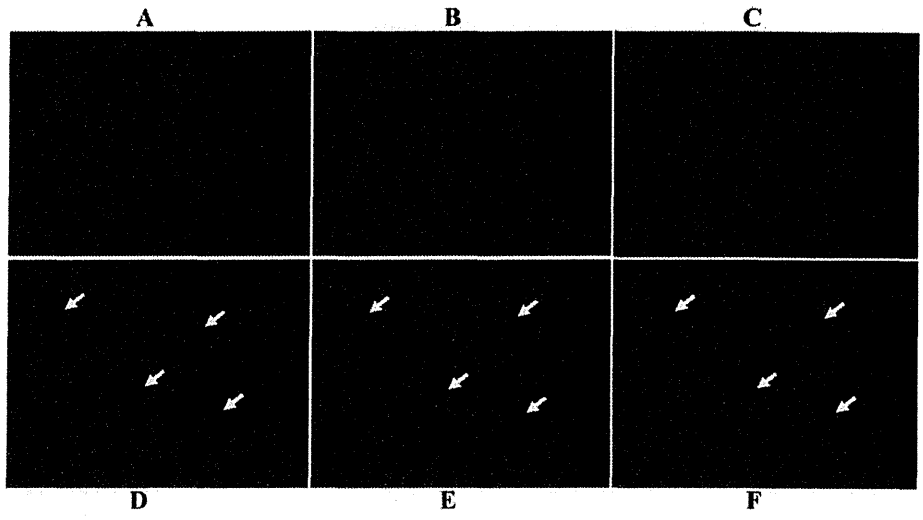

Figure 5 Double immunohistochemistry using chronic viral hepatitis livers. (A, B, C) Monocyte chemoattractant protein-1 receptor (CCR2, green), α-smooth muscle actin (red), and merged image, respectively. Double positive cells indicating CCR2-positive hepatic stellate cells (yellow) are not found. (D, E, F) CCR2 (green), CD68 (red) and merged image, respectively. Double positive cells indicating CCR2-positive Kupffer cells (yellow) are scattered (arrows).

BECs, only the expression of MCP-1 was upregulated, by two proinflammatory cytokines (by IL-1β and, to a lesser degree, by TNF-α) and all TLR ligands in an NF-κB-dependent manner. Because human BECs produced IL-1β when stimulated with TLR ligands,[15] the biliary innate immune response is suggested to be a critical trigger of MCP-1 production.

Bile ductules and their proliferation occur non-specifically in various hepatobiliary diseases. In normal livers, a few bile ductules are recognisable in the portal tract, while in various hepatobiliary diseases, these ductular structures are often increased in number, to be termed 'ductular proliferation' or 'proliferating bile ductules.' Because ductular proliferation was found accompanying interface hepatitis and periductal fibrosis in chronic liver diseases, these biliary elements are thought to take part in disease progression. However, the exact association between ductular proliferation and hepatic fibrogenesis is still unknown. Immunohistochemistry revealed that MCP-1 was expressed in bile ductules in areas of interface hepatitis, whereas normal livers lacked these findings. The ductular reaction predominantly corresponded to interface hepatitis in various chronic hepatobiliary diseases, and these areas were rich in several cytokines caused by immune-mediated (necro)inflammatory reactions against virus-infected hepatocytes and bile-derived PAMPs. Therefore, these microenvironments are suitable for the production of MCP-1 in proliferating bile ductules at the interface.

In damaged liver, HSCs are activated, proliferate and migrate into the injured area in response to the chemoattractive effects of chemokines. MCP-1 is a chemokine attracting monocyte/macrophages and plays a role in persistent inflammation in chronic liver diseases. Marra *et al*[3] reported that MCP-1 attracts HSCs, particularly activated HSCs, to the liver. Innate immunity is known to promote liver fibrosis, and as its mechanism, HSCs are reported to produce MCP-1 via TLR9 signalling.[16] In this study, immunohistochemistry using liver sections from patients with CVH and PBC revealed that αSMA-positive activated HSCs (myofibroblasts) were scattered and accumulated around MCP-1-expressing bile ductules in areas showing interface hepatitis. This finding suggests that MCP-1 derived from BECs plays a role in the chemoattraction of HSCs.

A recent report demonstrated that hepatocytes are also a source of MCP-1 and that hepatocyte-derived MCP-1 induced by a hydrophobic bile acid, taurocholate, chemoattracts HSCs and is associated with the liver fibrosis under cholestatic conditions in cases of paediatric cholestatic liver disease such as biliary atresia. Our immunohistochemical study also confirmed the expression of MCP-1 in periportal hepatocytes, but, in the early stage of CVH and PBC, the MCP-1 derived from bile ductules is speculated to the major effecter, compared with that from hepatocytes, based on the intensity of MCP-1 immunostaining. We also examined the effect of bile acids on the production of MCP-1 in cultured BECs. No bile acids affected the MCP-1 expression in BECs, suggesting that cholestasis could not directly induce the production of MCP-1 in BECs, differing from hepatocytes.

Portal fibroblasts located in portal tracts are fibrogenic cells distinct from HSCs and may be important mediators of biliary fibrosis and cirrhosis. Recently, Kruglov *et al*[17] reported that portal fibroblasts express functional receptors for MCP-1 that are distinct from CCR2 and that the secretion of MCP-1 by BECs induces myofibroblastic transdifferentiation of portal fibroblasts. In fact, the expression of MCP-1 was found in some interlobular bile ducts as well as bile ductules within portal tracts in CVH and PBC (data not shown). This finding suggests that MCP-1-dervied from BECs of these interlobular bile ducts is associated with the migration and activation of portal fibroblasts. However, we speculate that the fibrogenesis associated with portal fibroblasts is mainly associated with the histogenesis of portal sclerosis and expansive fibrous enlargement of portal tracts, not the fibrous extension accompanying interface hepatitis from portal tracts. The close correlation between MCP-1-positive bile ductules and αSMA-positive activated HSCs in the interface area shown in this study supports our contention. Moreover, we examined CHF as a control diseased liver in this study. CHF is different from cirrhosis in which no abnormal biliary channels are seen. In portal tracts of CHF, irregular and newly proliferating bile ducts and ductules rather than congenitally abnormal ductal plates, and cholestasis in these bile ductules are found. However, in parenchyma, the features of chronic cholestasis and interface changes are not prominent. Our previous study reported that the fibrogenesis of CHF is associated with intraportal heparan sulfate proteoglycan and CTGF, but not periportal HSCs.[14] It is true that although proliferating bile ductules were scattered within portal tracts, MCP-1 expression in bile ductules and αSMA-positive HSCs were not found in CHF, suggesting that the MCP-1-mediated migration of HSCs limited in proliferating bile ductules accompanying interface hepatitis of chronic inflammatory hepatobiliary diseases,

Take-home messages

- Biliary epithelial cells express several fibrogenic cytokines (MCP-1, PDGF-B, CTGF, TGF-β1 and endothelin-1), but only MCP-1 expression is upregulated by biliary innate immune reaction and proinflammatory cytokines (IL-1β and TNF-α).
- Proliferating bile ductules in interface areas expressed MCP-1 in diseased livers accompanying αSMA-positive activated hepatic stellate cells.
- MCP-1 derived from biliary epithelial cells plays an important role in the recruitment of hepatic stellate cells to interface areas and the activation of HSCs resulting in the progression of periportal fibrosis.

but not associated with the fibrogenesis in sole cholestatic liver diseases lacking interface changes such as CHF.

Because the receptor of MCP-1, CCR2, is not expressed in human HSCs, HSC migration by MCP-1 occurs independent of CCR2 via an unknown receptor for MCP-1, instead of by CCR2.[3] In fact, double immunohistochemistry in this study revealed that the expression of CCR2 is found not in αSMA-positive HSCs, but in CD68-positive Kupffer cells, suggesting that MCP-1-dervied from BECs could chemoattract Kupffer cells. As mentioned, portal fibroblasts within portal tracts also lack CCR2, but could be attracted by MCP-1 derived from BECs in a CCR2-independent manner.[17] In contrast, Seki *et al*[18] reported that both Kupffer cells and HSCs express CCR2 in mice but that these differences might be explained by differences between humans and mice.

Various types of inflammation including infection-triggered inflammation are causative factors to induce hepatic fibrosis in chronic liver diseases. Particularly, HSCs are critical for hepatic fibrogenesis, and MCP-1 is an important cytokine associated with HSC migration in fibrogenic areas. Therefore, the identification of MCP-1-producing cells and the clarification of the mechanism of MCP-1 production are mandatory to help regulate hepatic fibrosis and treat liver fibrosis. This study revealed that BECs are a source of MCP-1 in some hepatobiliary diseases, and the production of MCP-1 is induced by inflammatory cytokines and biliary innate immune responses. Proliferating bile ductules are thought to be part of a non-specific reaction in various hepatobiliary diseases, but this study suggests that they are closely associated with the progression of periportal fibrosis via MCP-1 derived from biliary innate immunity. Because MCP-1 is thought to be a key mediator of hepatic fibrosis, it is a potential therapeutic target in inflammatory hepatobiliary diseases with hepatic fibrosis.

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Competing interests None.

Patient consent Obtained.

Ethics approval This study was approved by the Kanazawa University Ethics Committee.

Contributors KH and YN conceived and carried out the experiments; AO, MH, YS, SI, XSR, HI, HO, SK and AK conceived the experiments and analysed data; MC carried out the experiments. All authors were involved in writing the paper and had final approval of the submitted and published versions.

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Mucinous cystic neoplasms of the liver: a clinicopathological study and comparison with intraductal papillary neoplasms of the bile duct

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Mucinous cystic neoplasm of the liver has been a controversial entity, in particular, regarding differentiation from intraductal papillary neoplasm of the bile duct. In this study, we compared the characteristics of hepatic mucinous cystic neoplasms with ovarian-like stroma ($n=29$) to those of cyst-forming intraductal papillary neoplasms of the bile duct ($n=12$). Radiological or macroscopic appearance, histological grade of malignancy, and postoperative clinical course were recorded. Immunohistochemistry for biliary or gastrointestinal markers was performed to characterize cell phenotypes. The patients with hepatic mucinous cystic neoplasm were all female and ranged in age from 21 to 67 years, which was significantly younger than that in the patients with biliary intraductal papillary neoplasm. Eighteen mucinous cystic neoplasms (76%) were located in the left lobe, with 13 (54%) in segment IV. Mucinous cystic neoplasms were significantly larger than intraductal papillary neoplasms (median diameter: 110 *vs* 50 mm, $P=0.008$). In contrast to intraductal papillary neoplasms that were all histologically malignant, 26 mucinous cystic neoplasms (90%) were adenomas, 2 (7%) were borderline malignant, and 1 (3%) was a carcinoma *in situ*. Benign mucinous cystadenomas had the pure biliary immunophenotype, whereas gastrointestinal markers including cytokeratin 20 and mucin core proteins 2, 5AC, and 6 were more frequently expressed in borderline or malignant mucinous cystic neoplasms and biliary intraductal papillary neoplasms. There was no mortality in the patients with mucinous cystic neoplasm, whereas one patient with intraductal papillary neoplasm died of cancer. In conclusion, hepatic mucinous cystic neoplasms and biliary intraductal papillary neoplasms have different clinicopathological characteristics as evidenced by differences in the age and gender of patients, macroscopic appearance, immunophenotypes, and grades of malignancy.
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Keywords: cyst; cholangiocarcinoma; dysplasia; intestinal metaplasia; liver

Mucinous cystic neoplasms of the hepatobiliary system, also called biliary cystic neoplasms, are rare tumours usually developing within the liver.[1] Clinicopathological characteristics of hepatic mucinous cystic neoplasm have not been elucidated because pathological diagnostic criteria were not clear.[1] The most recent WHO classification defined the hepatic mucinous cystic neoplasms as a cyst-forming epithelial neoplasm, usually showing no communication with the bile ducts, composed of cuboidal to columnar, variably mucin-producing epithelium, and associated with ovarian-type

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subepithelial stroma.[2] Mucinous cystic neoplasms need to be distinguished from other cystic liver lesions including simple cysts and cystic hamartomas.[3] Recently, a unique biliary tumour named intraductal papillary neoplasm of the bile duct has been included among biliary cystic tumours.[4] Biliary papillomas, biliary papillomatosis, and some of papillary cholangiocarcinomas are included in this category. Biliary intraductal papillary neoplasm is considered as a biliary counterpart of intraductal papillary mucinous neoplasms of the pancreas based on a predominant intraductal growth, ductal dilatation, and occasional overproduction of mucin.[4] A previous study revealed that cystic liver tumours with bile duct communication share characteristics with intraductal papillary neoplasms, but not mucinous cystic neoplasms.[5]

There had been controversy regarding the distinction between mucinous cystic neoplasms and intraductal papillary neoplasms until the presence of an ovarian-like stroma was accepted as a prerequisite for the diagnosis of mucinous cystic neoplasms and the distinct entity of biliary intraductal papillary neoplasms was proposed. Most studies to date have likely examined both types of cystic tumours under a single tumour entity.[6–8] Similar confusion regarding pancreatic lesions existed several decades ago.[9] However, two distinct entities, pancreatic mucinous cystic neoplasms and intraductal papillary mucinous neoplasms, are now widely accepted.[10]

In this study, the clinicopathological characteristics of hepatic mucinous cystic neoplasms are compared with those of biliary intraductal papillary neoplasms.

Patients and methods

Pathological Material

A total of 29 surgical cases of hepatic mucinous cystic neoplasm were retrieved from the histopathology files of the Institute of Liver Studies at King's College Hospital ($n=14$), University of Verona ($n=12$), and Kanazawa University Hospital ($n=3$), during the period 1991–2010. Twelve cases of cystic-type intraductal papillary neoplasm of the bile duct were also retrieved from the histopathology files of King's College Hospital, Kanazawa University Hospital, and the consultation files of one of the authors (YZ). Four of the intraductal papillary neoplasms were used in a previous study.[5] Five surgical specimens with simple biliary cysts and 10 liver specimens with normal bile ducts resected for metastatic colorectal cancer were used as controls for immunohistochemistry.

The diagnosis of mucinous cystic neoplasms was based on the presence of an ovarian-like stroma in the cyst wall. Cystic-type intraductal papillary neoplasm was diagnosed on the basis of intraductal non-invasive high papillary proliferation with intraepithelial extension as described in previous studies.[5,11] All cases of intraductal papillary neoplasm showed a predominant cystic appearance on imaging. No patients had a history of chronic cholangiopathy such as intrahepatic stones or primary sclerosing cholangitis.

Radiological or Macroscopic Examination

Radiological or macroscopic features were reviewed, in particular the location (intrahepatic or hilar area; caudate lobe, left lateral segment, left medial segment (segment IV), or right lobe) and shape (unilocular, multilocular, or multicystic) of the lesion. Multilocular cysts were defined as a single cyst with multiple spaces divided by septa or showing a cyst-in-cyst appearance, the cyst outline remaining smooth. The definition of multicystic lesions was an accumulation of multiple cysts with an indented outline producing a grape-like appearance. Representative images and schema are shown in Figure 1. The presence or absence of papillary mural nodules was also recorded.

Pathological Examination

The pathological features assessed included histological grade of malignancy, tumour cell morphology, presence or absence of ovarian-like stroma, and extension into adjacent bile ducts. Histological grading was based on the basis of the highest degree of cytoarchitectural dysplasia with low-grade (adenoma), intermediate-grade (borderline malignancy), and high-grade (carcinoma *in situ*).[2,12,13] The presence or absence of an associated invasive cancer was also recorded.

Immunohistochemistry

We examined cell phenotypes using nine proteins: cytokeratin (CK) 7, CK20, mucin core protein (MUC) 1, MUC2, MUC5AC, and MUC6 for biliary epithelium, and oestrogen receptor, progesterone receptor (PgR), and inhibin α for stromal cells. One representative section selected from each case was used for immunostaining. Immunostaining of the nine proteins was carried out using the EnVision + system (Dako Cytomation, Glostrup, Denmark). The deparaffinized sections were microwaved in EDTA buffer, pH 9.0, for 5 min twice at a 2-min interval. After the blocking of endogenous peroxidase by REAL Peroxidase-Blocking Solution (Dako Cytomation), the deparaffinized sections were incubated for 1 h at room temperature with primary monoclonal antibodies: anti-CK7 (clone OV-TL 12/30; 1:200; Dako Cytomation), anti-CK20 (clone Ks20.8; 1:100; Dako Cytomation), anti-MUC1 (clone DF3; 1:50; Abcam, Cambridge, UK), anti-MUC2 (clone Ccp58; 1:100; Novocastra Laboratories, Newcastle, UK),

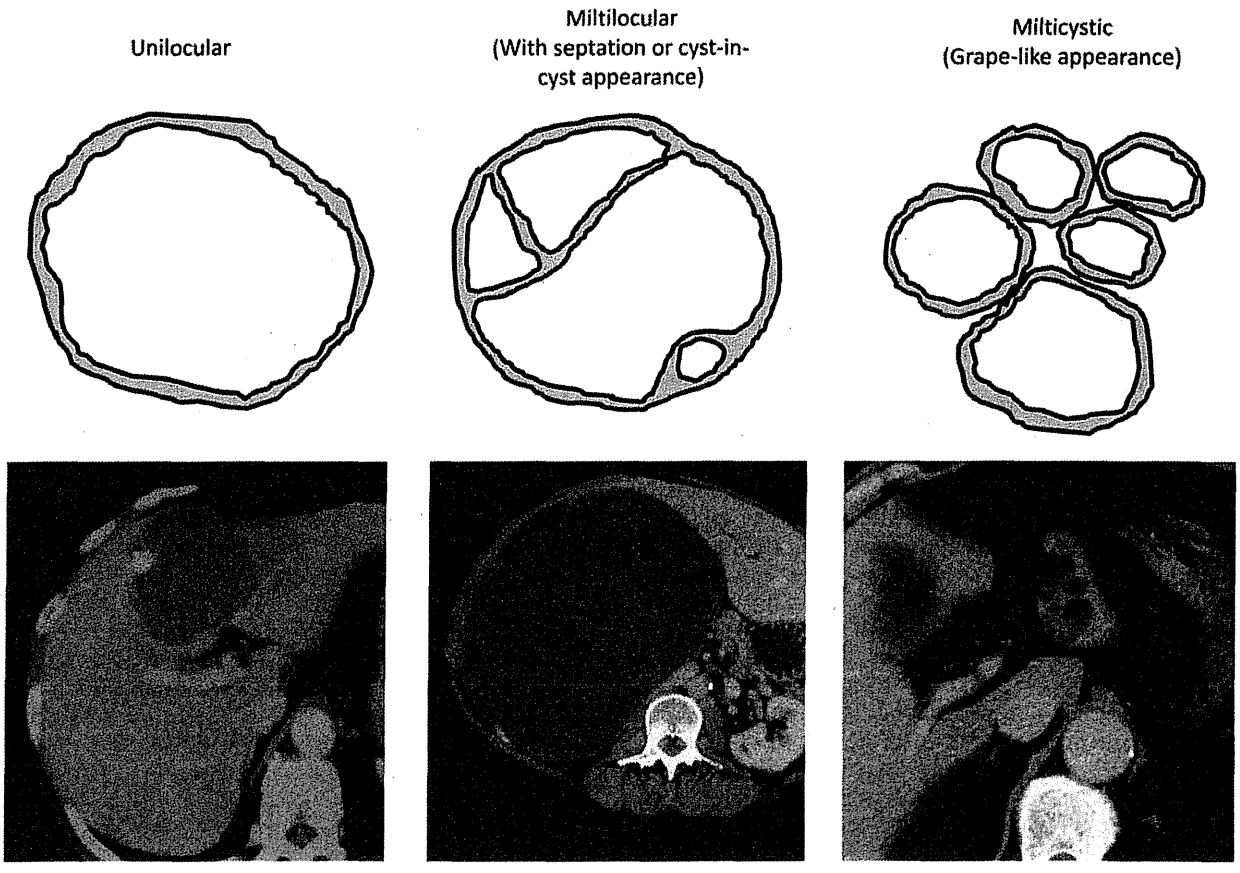

Figure 1 Radiological/macroscopic appearance of cystic tumours.

anti-MUC5AC (clone CLH2; 1:100; Novocastra Laboratories), anti-MUC6 (clone CLH5; 1:100; Novocastra Laboratories), anti-ER (clone 1D5; 1:100; Dako Cytomation), anti-PgR (clone 16; 1:40; Novocastra Laboratories), and anti-inhibin α (clone R1; 1:50; Dako Cytomation). The sections were then incubated at room temperature for 1 h with goat anti-mouse immunoglobulins conjugated to a peroxidase-labelled dextran polymer (EnVision+; Dako Cytomation). The reaction products were developed by immersing the section in a 3,3′-diaminobenzidine tetrahydrochloride solution. Nuclei were lightly counterstained with hematoxylin. The expression was evaluated semiquantitatively according to the percentage of positive epithelial or mesenchymal cells in an individual lesion: 0 (negative); 1+ (focal), 1–10%; 2+ (moderate), 11–50%; and 3+ (marked), more than 50%. Cases with moderate or marked expression were considered positive.

Statistical Analysis

Statistical analyses were performed using the χ^2 or Mann–Whitney *U*-test for two groups or Tukey's test for more than two groups. A probability of $P<0.05$ was considered statistically significant.

Table 1 Gender and age of patients with hepatic mucinous cystic neoplasm or biliary intraductal papillary neoplasm

	Mucinous cystic neoplasm (n=29)	*Intraductal papillary neoplasm (n=12)*	P-*value*
Gender (M/F)	0/29	6/6	**<0.001**
Age (median, range)	45 (21–69)	62 (43–77)	**0.006**
Age, <50 years	16 (55%)	1 (8%)	**0.016**

Bold values indicate significant difference.

Results

Clinical Features

All patients with mucinous cystic neoplasm were female, ranging in age from 21 to 69. They were characterized by a higher ratio of female patients and younger age compared to the patients with intraductal papillary neoplasm (Table 1). Sixteen of

29 patients (55%) with mucinous cystic neoplasm were under 50 years, whereas 11 of 12 patients (92%) with intraductal papillary neoplasm were over 50 years. The three patients with borderline or malignant mucinous cystic neoplasm were older than the 26 patients with benign mucinous cystadenoma (median age, 60 *vs* 44, $P=0.049$).

Radiological or Macroscopic Findings

Radiological or macroscopic findings were reviewed for 26 patients with mucinous cystic neoplasm and 10 patients with intraductal papillary neoplasm. There were no available records for five earlier cases of mucinous cystic neoplasm ($n=3$) and intraductal papillary neoplasm ($n=2$) and data were available only after a partial cystectomy for one case of mucinous cystic neoplasm. Mucinous cystic neoplasms were larger than intraductal papillary neoplasms ($P=0.008$). The left lobe was prevalent for both tumours, with 54% of mucinous cystic neoplasms located in the left medial segment (segment IV) (Table 2). In terms of the shape of the lesion, 21 of 26 (81%) mucinous cystic neoplasms showed multilocular cysts with septation or a cyst-in-cyst appearance. In contrast, 8 of 10 (80%) intraductal papillary neoplasms were multicystic with a grape-like appearance. The partially excised mucinous cystic neoplasm showed a multicystic appearance. Papillary mural nodules were noted in all intraductal papillary neoplasms (100%), but in only one mucinous cystic neoplasm (4%), the one with malignant features ($P<0.001$). The sensitivity and specificity of using the appearance of multilocular cysts for the diagnosis of mucinous cystic neoplasm were 81% and 95%, respectively. A multicystic appearance is highly sensitive (80%) and specific (80%) for the diagnosis of intraductal papillary neoplasm, a multicystic appearance with mural nodules showing 100% specificity for intraductal papillary neoplasm.

Pathological Features

Mucinous cystic neoplasms

All mucinous cystic neoplasms had a single layered epithelium supported by fibrous connective tissue. Twenty-six cases (90%) were adenomas consisting of minimally atypical biliary-type epithelium with occasional mucin-containing cells (Figures 2a and b). In two cases (7%), there were atypical foci showing enlarged nuclei, nuclear stratification, and an increased nuclear/cytoplasmic ratio, but no cytological atypia of a carcinoma, and the tumours were graded as borderline malignant (Figures 2c and d). These atypical foci were histologically flat or micropapillary, and found incidentally by microscopic examination. The remaining case (3%) was of carcinoma *in situ* with a marked papillary proliferation of atypical epithelium forming a complex papillotubular glandular architecture, associated with increased cellularity, nuclear pleomorphism, and occasional mitotic figures (Figure 3). Benign or borderline components were seen in adjacent flat areas.

Table 2 Radiological/macroscopic features of hepatic mucinous cystic neoplasms and biliary intraductal papillary neoplasms

	Mucinous cystic neoplasm (n = 26)[a]	*Intraductal papillary neoplasm* (n = 10)[a]	P-*value*
Size (median, range)	110 mm (25–250)	50 mm (25–140)	**0.008**
Location			
Intrahepatic	24 (92%)	9 (90%)	0.825
Hilar	2 (8%)	1 (10%)	
Lobe or segment[b]			
Caudate lobe	1 (4%)	1 (10%)	0.777
Left lateral segment	5 (21%)	3 (30%)	
Left medial segment	13 (54%)	3 (30%)	
Right lobe	5 (21%)	3 (30%)	
Shape			
Unilocular	3 (11%)	1 (10%)	**<0.001**
Multilocular	21 (81%)	1 (10%)	
Multicystic	2 (8%)	8 (80%)	
Papillary mural nodule			
Presence	1 (4%)[c]	10 (100%)	**<0.001**
Absence	25 (96%)	0	

[a]No record available in five old cases (three mucinous cystic neoplasms and two intraductal papillary neoplasms).
[b]Two mucinous cystic neoplasms in hilar tissue could not be further located.
[c]Carcinoma *in situ* case.
Bold values indicate significant difference.

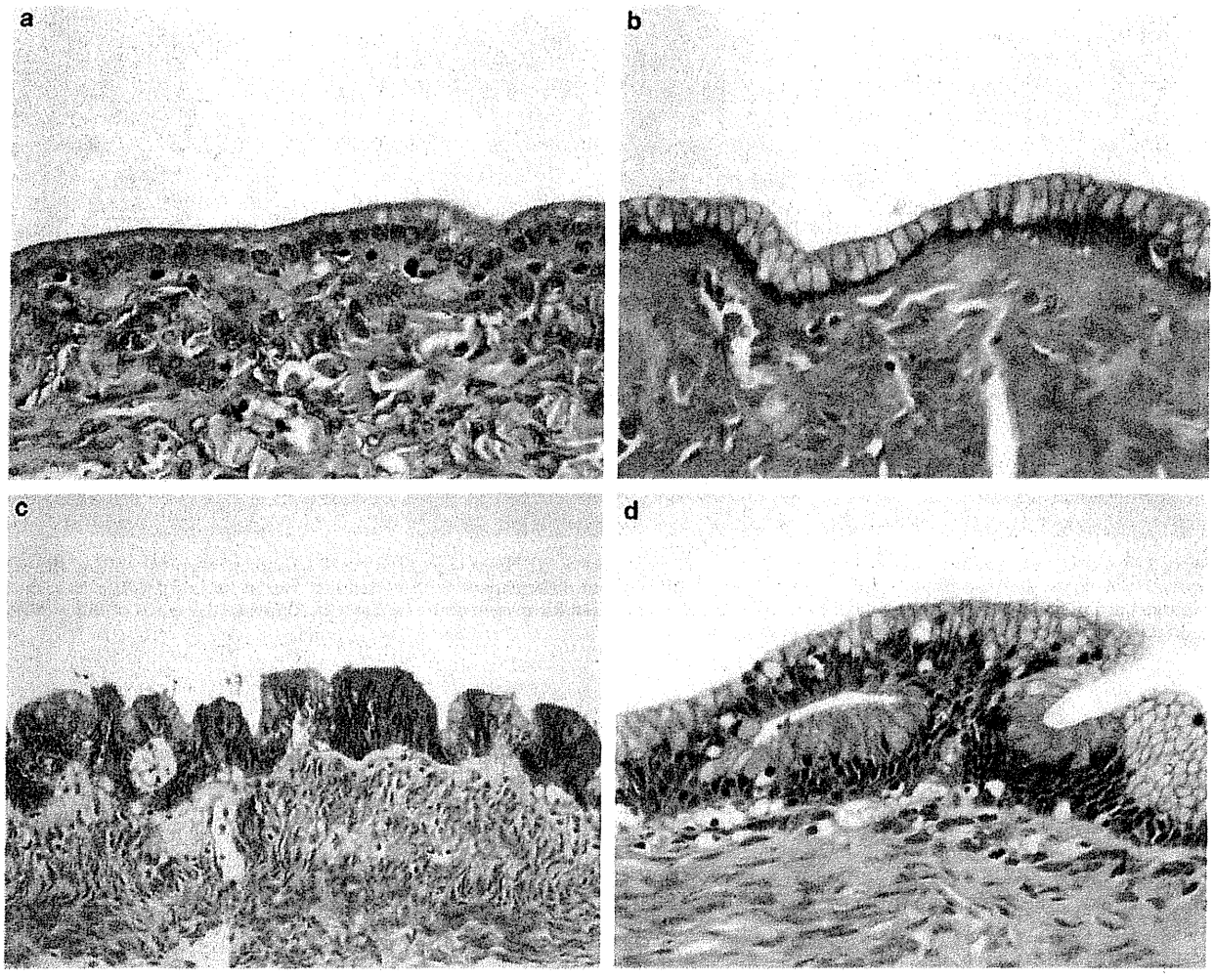

Figure 2 Histopathology of hepatic mucinous cystic neoplasms. Benign mucinous cystadenomas show a single layered biliary-type epithelium (**a**) with occasional foci of mucin-containing cells (**b**). Borderline tumours exhibit cellular atypia such as nuclear enlargement and stratification (**c, d**). Original magnification: **a**, **b**, **d**, ×400; **c**, ×200.

An ovarian-like stroma consisting of cellular spindle cells was easily and relatively diffusely identified in all cases. In the case of carcinoma *in situ*, the stroma was also noted in papillary areas (Figure 3). Haemosiderin deposition was noted in 13 cases, cholesterol clefts in nine cases, and small foci of calcification in five cases. Features suggestive of ductal communication such as the presence of peribiliary glands in the cyst wall or the presence of hepatic parenchyma entrapped in the interlocular septa were not identified.

Comparison with intraductal papillary neoplasms
All intraductal papillary neoplasms showed extensive papillary proliferation of atypical epithelium within the cystic space (Figures 4a and b). Ten cases of intraductal papillary neoplasms (83%) were graded as carcinoma *in situ*, and the remaining two case had foci of invasion (microinvasive mucinous carcinoma in one and invasive tubular adenocarcinoma in the other). In all intraductal papillary neoplasms, bile duct communication was evidenced by the presence of intraepithelial neoplasms within adjacent bile ducts, or the presence of peribiliary glands in the cyst wall (Figures 4c and d). No ovarian-like stroma was found in any cases of intraductal papillary neoplasm (Table 3).

Immunohistochemistry

Normal bile ducts
Normal bile ducts had the immunophenotype $CK7^+$/$CK20^-$/$MUC1^-$/$MUC2^-$/$MUC5AC^-$. MUC6 was expressed in peribiliary glands in two cases.

Simple cysts
The surface epithelium of simple cysts showed the immunophenotype $CK7^+$/$CK20^-$/$MUC1^-$/$MUC2^-$/

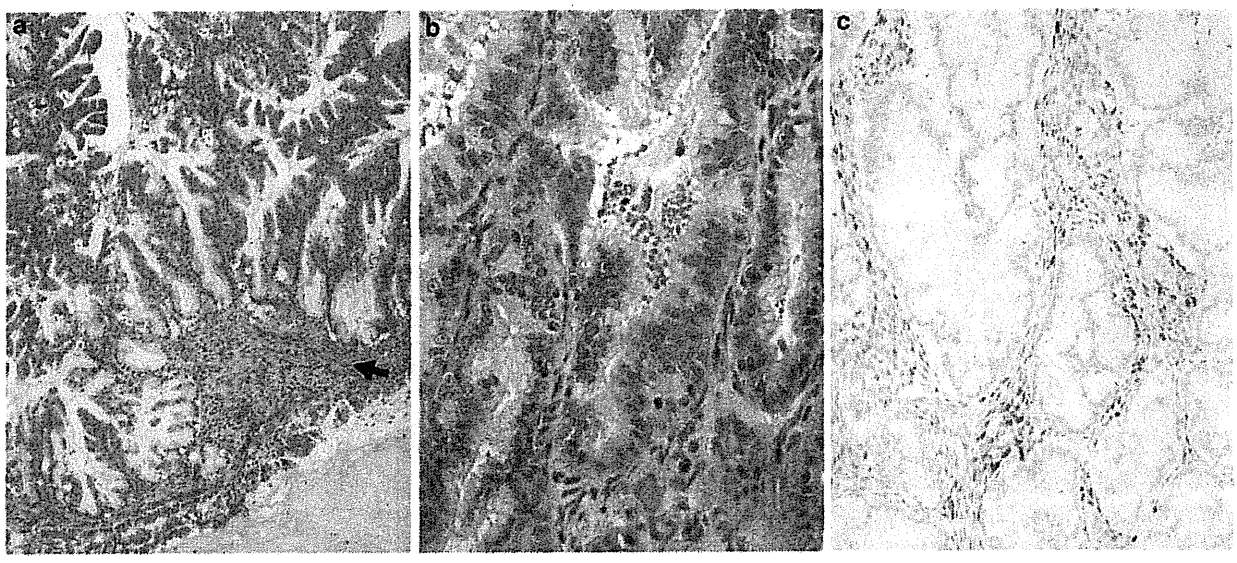

Figure 3 A carcinoma *in situ* case of hepatic mucinous cystic neoplasm. (**a**) There is papillary proliferation of atypical epithelium with fibrovascular cores. Ovarian-like stroma is noted (arrow). (**b**) Nuclear enlargement with occasional foci of loss of polarity is seen. (**c**) Immunostaining for the progesterone receptor reveals a stroma positive for progesterone receptor in fibrovascular cores of the papillary lesion. Original magnification: **a**, ×100; **b**, **c**, ×400.

MUC6$^-$. MUC5AC expression was observed in one case (20%).

Mucinous cystic neoplasms
All benign mucinous cystadenomas ($n=26$) showed the biliary immunophenotype CK7$^+$/CK20$^-$/MUC2$^-$/MUC5AC$^-$/MUC6$^-$ (Figure 5). The expression of MUC2 and MUC5AC was focally observed in 11 and one adenoma, respectively (less than 2% of tumour cells). Gastrointestinal markers such as CK20, MUC2, MUC5AC, and MUC6 were more frequently expressed in borderline or malignant tumours than benign mucinous cystadenomas (all, $P<0.001$) (Table 4 and Figures 5). MUC2 and MUC5AC were expressed in all borderline and malignant cases. MUC2 positivity was restricted to goblet cells. Moderate to marked expression of MUC1 was observed only in the carcinoma *in situ* case (Figure 5). Regarding the ovarian-like stroma, ER, PgR, and inhibin α were expressed in 19 (67%), 27 (93%), and 6 (20%) cases, respectively (Figure 3c).

Intraductal papillary neoplasms
The expression of CK7 was decreased in two cases: a very focal expression in one case and no expression in the other. Intestinal markers (CK20 and MUC2) were expressed in 2 (17%) and 5 (42%) cases, respectively. Gastric markers (MUC5AC and MUC6) were expressed in most cases. Moderate to marked expression of MUC1 was observed in 6 (50%) cases, including invasive cases. No expression of ER, PgR, and inhibin α was seen in any cases.

Comparison between mucinous cystic neoplasms and intraductal papillary neoplasms
The expression of CK7 was less common in intraductal papillary neoplasms, whereas the expression of MUC1, MUC2, MUC5AC, and MUC6 was significantly more common in intraductal papillary neoplasms (Table 4). The expression of gastrointestinal markers was similarly common in borderline or malignant mucinous cystic neoplasms and intraductal papillary neoplasms. In contrast, benign mucinous cystadenomas had a pure biliary immunophenotype. Moderate to marked expression of MUC1 was only observed in malignant cases in both types of tumours.

Recurrence

Follow-up data were reviewed for 24 patients with mucinous cystic neoplasm and 12 patients with intraductal papillary neoplasm. No records were available for five earlier cases of mucinous cystic neoplasm. All the patients with mucinous cystic neoplasm were treated surgically, and none died of this tumour (follow-up period, 1–132 months, median 47 months). Eighteen (75%) patients were treated by single surgical resection. Two (8%) patients, including the one with the carcinoma *in situ*, first underwent a partial cystectomy with fenestration in referring hospitals before undergoing a complete excision. One additional patient (4%) underwent a left hepatectomy for a large tumour predominantly in segment IV. Parts of the tumour remained in the right lobe, but have not changed in

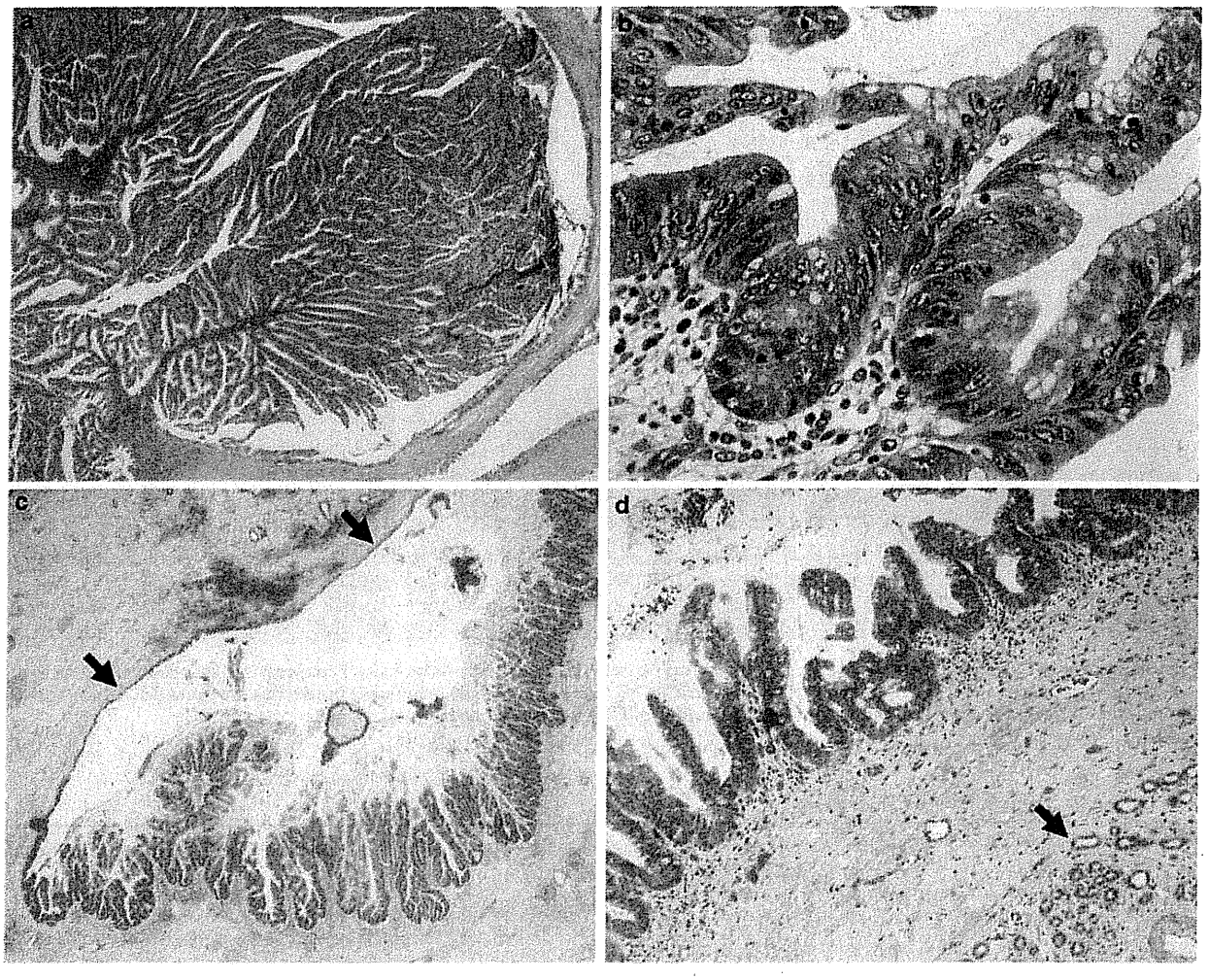

Figure 4 Histopathology of biliary intraductal papillary neoplasms. **(a)** There is a papillary tumour in the cystic space. **(b)** The tumour consists of large atypical cells with occasional intracytoplasmic mucin. **(c)** An intraductal extension of the tumour is noted in a large bile duct. Arrows indicate non-neoplastic biliary epithelium. **(d)** Peribiliary glands are seen in the cyst wall (arrow). Original magnification: **a**, **c**, ×20; **b**, ×400; **d**, ×100.

Table 3 Pathological features of hepatic mucinous cystic neoplasms and biliary intraductal papillary neoplasms

	Mucinous cystic neoplasm (n=29)	*Intraductal papillary neoplasm (n=12)*	P-*value*
Grading			
Adenoma	26 (90%)	0	**<0.001**
Borderline	2 (7%)	0	
Malignancy	1 (3%)	12 (100%)	
Ductal communication			
Presence	0	12 (100%)	**<0.001**
Absence	29 (100%)	0	
Ovarian-like stroma			
Presence	29 (100%)	0	**<0.001**
Absence	0	12 (100%)	

Bold values indicate significant difference.

appearance or size over a follow-up period of 6 years and 9 month. The remaining three (13%) patients (all benign cases) were radiologically found to have a recurrent mucinous cystic neoplasm (5 months, 7 years, and 8 years and 7 months after the first operation). Interestingly, one recurrent mucinous

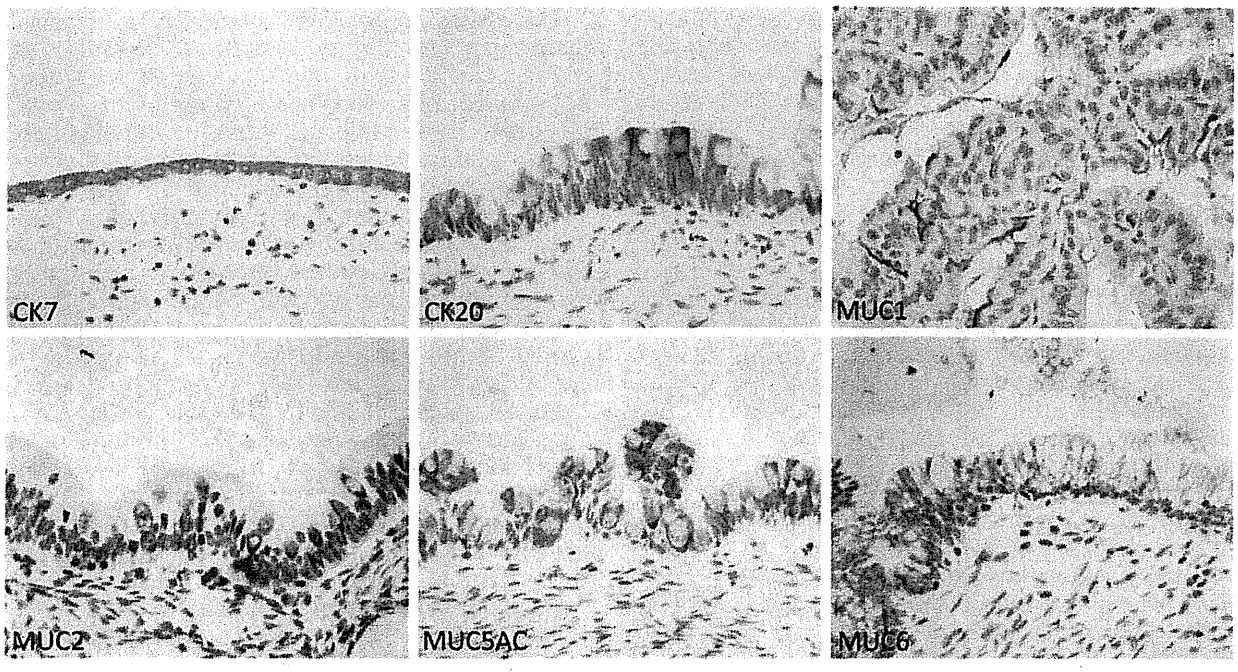

Figure 5 Immunohistochemistry in hepatic mucinous cystic neoplasms. Cytokeratin (CK) 7 expression is observed in a benign cystadenoma. Expression of CK20, mucin core protein (MUC)2, MUC5AC, and MUC6 is noted in borderline tumours. MUC1 is expressed on apical membrane of a malignant mucinous cystic neoplasm. Original magnifications: all, ×400.

Table 4 Results of immunohistochemistry

	Normal bile duct (n=10)	*Simple cyst* (n=5)	*Mucinous cystic neoplasm* (n=29)	*Intraductal papillary neoplasm* (n=12)	P-value[a]	*Mucinous cystic neoplasm*		P-value[b]
						Benign (n=26)	*Borderline to CIS* (n=3)	
CK7	10 (100%)	10 (100%)	29 (100%)	10 (83%)	0.276	26 (100%)	3 (100%)	Identical
CK20	0	0	2 (7%)	2 (17%)	0.703	0	2 (67%)	**0.002**
MUC1	0	0	1 (3%)	6 (50%)	**0.002**	0	1 (33%)	0.185
MUC2	0	0	2 (7%)	5 (42%)	**0.025**	0	2 (67%)	**0.002**
MUC5AC	0	1 (20%)	3 (10%)	12 (100%)	**<0.001**	0	3 (100%)	**<0.001**
MUC6	2 (20%)[c]	0	2 (7%)	10 (83%)	**<0.001**	0	2 (67%)	**0.002**

CIS, carcinoma *in situ*.
[a]Between MCN and IPNB.
[b]Between benign and borderline to CIS.
[c]Expression in peribiliary glands.
Bold values indicate significant difference.

cystic neoplasm had increased in size at the time of pregnancy. All resected recurrent tumours were cystadenoma without malignant transformation.

All patients with intraductal papillary neoplasm except one were treated by complete surgical resection. No recurrence has been observed during a median follow-up period of 44 months. A complete resection was impossible in the remaining patient with a widely invasive tumour. This patient died of Budd–Chiari syndrome due to carcinoma invasion into vena cava.

Discussion

The clinicopathological findings characteristic of hepatic mucinous cystic neoplasms can be summarized as follows: (1) patients are female and range widely in age; (2) multilocular cysts with septation or a cyst-in-cyst appearance are distinctive; (3) 90% of cases are histologically benign; (4) a gastrointestinal immunophenotype seems important to the histological progression; and (5) although local recurrence rarely occurs, at times years after surgery,

the prognosis is excellent. There has been controversy regarding the distinction between hepatic mucinous cystic neoplasms and biliary intraductal papillary neoplasms, but this study reveals clinicopathological differences that are sufficient to support that these tumours are two distinct entities.

In the largest study of pancreatic mucinous cystic neoplasm to date, 155 of 163 (95%) patients were female, and ranged in age from 16 to 82.[14] These demographic data are similar to those for hepatic mucinous cystic neoplasm, except for rare cases of pancreatic cystadenoma occurring in men. Almost all pancreatic mucinous cystic neoplasms (97%) were located on the left side (the body-tail) of the pancreas.[14] Hepatic mucinous cystic neoplasms were similarly common on the left side (75%). The postoperative survival rate was excellent for both hepatic and pancreatic mucinous cystic neoplasms, with no mortality recorded in both studies.

However, there are several pathological differences between hepatic and pancreatic mucinous cystic neoplasms. The proportion of malignant tumours was higher in pancreatic cases. Of 163 patients with pancreatic mucinous cystic neoplasms, 118 (72%) were adenomas, 17 borderline neoplasms (10.5%), 9 carcinomas *in situ* (5.5%), and 19 were invasive carcinomas (12%).[14] Lüttges *et al*[15] reported that MUC2 expression in pancreatic mucinous cystic neoplasm was confined to goblet cells, which were numerous in borderline or malignant cases, but rare in adenomas. This is in line with our results in hepatic mucinous cystic neoplasms. In contrast, MUC5AC and CK20 were expressed in the majority of pancreatic mucinous cystic neoplasms irrespective of the grade of dysplasia,[15,16] which differs markedly from the immunophenotype found in our study.

Of interest, only one (3%) hepatic mucinous cystic neoplasm was malignant, a rate quite low compared with previous reports. Studies not using the ovarian-like stroma as a diagnostic criterion found the ratio of malignant cases of biliary cystic tumours to be 21–26%.[6,7] Ovarian-like stroma was less commonly observed in the malignant cases in these studies.[6,7] Presumably, cases of cystic-type intraductal papillary neoplasm were included in the malignant groups in these studies, given that almost all cases of biliary intraductal papillary neoplasm were at least carcinoma *in situ*. However, even in studies restricted to cystic tumours with ovarian-like stroma, 17–19% of tumours were malignant.[17,18] One possible explanation for this discrepancy is that owing to recent advances in imaging modalities, patients in this study were diagnosed at a younger age; patients with malignant hepatic and pancreatic mucinous cystic neoplasms were older than those with benign cystadenomas.[14,18] This may stress the importance to treat patients with hepatic mucinous cystic neoplasm at a young age to reduce the risk of malignant transformation.

This study suggested that hepatic mucinous cystic neoplasms and biliary intraductal papillary neoplasms can differ in radiological or macroscopic appearance. Lim *et al*[19] also reported that intraductal papillary neoplasms more commonly show peripheral bile duct dilatation than mucinous cystic neoplasms. However, the clinical diagnosis of biliary cystic lesions remains challenging, due to other hamartomatous and developmental cystic lesions with or without cholangiocarcinoma, which may mimic mucinous cystic neoplasms.[20] In addition, about 10% of hepatic mucinous cystic neoplasms manifest as unilocular cystic lesions, which are difficult to differentiate from solitary simple cysts. Interestingly, two hepatic mucinous cystadenoma in this study appeared as unilocular cyst radiologically, but small intramural cysts were identified on macroscopic examination of the resected specimen and therefore categorized as multilocular cysts in this study. Further development of radiological modalities will be needed to detect such small mural cysts.

Two major pathways have been suggested for biliary carcinogenesis. More commonly, biliary intraepithelial neoplasia (biliary dysplasia) evolve into cholangiocarcinoma usually manifesting as an infiltrative tubular adenocarcinoma.[11,21] The other pathway, intraductal papillary neoplasm to invasive cancer, is characterized by a potential progression to both mucinous carcinoma and papillotubular carcinoma, the expression of gastrointestinal markers even in early lesions (adenomas), and a more favourable postoperative prognosis.[11,22] This study suggested that mucinous cystic neoplasm may be a tumour precursor through another route characterized by the least aggressive clinical course and by expression of gastrointestinal molecules during the carcinogenetic process.

Regarding the location of biliary cystic tumours, most mucinous cystic neoplasms arise in the liver, except for rare tumours in the gallbladder or common bile duct.[6,17] Biliary intraductal papillary neoplasm can arise in both extrahepatic and intrahepatic bile ducts, but most cyst-forming tumours affect hilar or intrahepatic bile ducts.[5] In our experience, the incidence of hepatic mucinous cystic neoplasm is higher than that of biliary intraductal papillary neoplasm in European countries. In contrast, biliary intraductal papillary neoplasm is more commonly seen in Asian countries partly because of a higher incidence of hepatolithiasis or Clonorchiasis infection,[11,23] whereas hepatic mucinous cystic neoplasm is extremely rare in Japan.

The origin of hepatic and pancreatic mucinous cystic neoplasms has not been elucidated. Mucinous cystic neoplasms may develop from endodermal immature stroma or primary yolk cells implanted during embryogenesis.[24,25] The prevalence of hepatic mucinous cystic neoplasm in segment IV may support an implant origin because hamartomatous

lesions commonly develop in segment IV.[26,27] The expression of oestrogen receptor or PgR in ovarian-like stroma also supports a putative role for female hormones in the tumorigenesis.[28]

In conclusion, hepatic mucinous cystic neoplasms and biliary intraductal papillary neoplasms have different clinicopathological characteristics as evidenced by differences in the age and gender of patients, macroscopic appearance, immunophenotypes, and grades of malignancy. It is rational to use the presence of ovarian-like stroma to differentiate hepatic mucinous cystic neoplasms from biliary intraductal papillary neoplasms.

Disclosure/conflict of interest

The authors declare no conflict of interests.

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Original Article

Pathological spectrum of intrahepatic cholangiocarcinoma arising in non-biliary chronic advanced liver diseases

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Intrahepatic cholangiocarcinoma (ICC) is reported to develop in non-biliary chronic advanced liver diseases (CALD). Herein, we characterize the pathological features of ICC arising in CALD in comparison with those in non-CALD livers. Of 471 surgically resected cases of ICC in Kanazawa, Japan and Seoul, Korea, 53 were associated with CALD (group A), while the remaining 418 arose in otherwiswe normal livers (group B). When ICC were classified into bile duct type, bile ductular type, variants, and intraductal papillary neoplasm of the bile duct (IPNB), the whole spectrum of subtypes were found in group A; the majority of ICC belonged to the bile duct type in both groups. In group A, bile ductular type was rather frequent (22.6%) compared with group B (8.4%). IPNB was more frequent in group B (22.5%) than group A (3.8%), and in group B, frequent in Seoul cases (24.8%), but rare in Kanazawa cases (2.3%). Variants of ICC were rare in both groups. These results imply that cholangiocarcinogenesis itself is upregulated in group A in comparison with group B and that the bile ductular type is specifically related to group A. Some unique environmental factors in Seoul may be responsible for the frequent development of IPNB.

Key words: intraductal papillary neoplasm, intrahepatic cholangiocarcinoma, liver cirrhosis, progenitor cells

Intrahepatic cholangiocarcinoma (ICC) is a malignant epithelial neoplasm arising from the intrahepatic biliary tree with features of cholangiocyte differentiation.[1–3] While ICC usually develops in an apparently normal liver, some cases are associated with long-standing biliary diseases such as hepatolithiasis and primary sclerosing cholangitis (PSC) as well as biliary tract anomalies such as Caroli's disease. There have been many studies on cholangiocarcinogenesis in long-standing biliary diseases, and chronic inflammation of the bile ducts with sustained stress on biliary epithelial cells (BEC) is reportedly at least partly responsible for cholangiocarcinogenesis.[1–4]

ICC is also known to develop in non-biliary, chronic advanced liver diseases (CALD) such as chronic viral hepatitis-related cirrhosis.[5–8] Epidemiological studies indicate that chronic infection with the hepatitis virus, particularly HCV and HBV, is a possible risk factor for ICC.[4,9–11] Shaib *et al.* reported that compared with controls, ICC patients had a higher prevalence of anti-HCV antibodies (6.0% vs. 0.8%) and anti-HBc antibodies (9.6% vs. 0%), and that the prevalence of cirrhosis was high in patients with ICC (24.1%) in the United States.[4] HCV infection is also reported to be a likely etiology of ICC in Japan.[5,6] In fact, patients with cirrhosis due to HCV had a 1000-fold higher risk of ICC than the general population.[10] Yamamoto *et al.* detected HCV seropositivity in 36% of ICC patients and 3% of controls, in a hospital-based case–control study.[6] The development of ICC seems also to be related to HBV infections in areas where both HBV and ICC are endemic.[12–14] To date, there have been only a limited number of pathological studies on ICC arising in non-biliary CALD, in which ICC reportedly showed a nodular growth pattern and were simply described as well-differentiated adenocarcinomas.[5–7]

In this study, we try to characterize the pathological features of ICC arising in non-biliary CALD in comparison with ICC arising in non-cirrhotic livers, using a large number of surgically resected cases of ICC in the Far East.

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METERIALS AND METHODS

Anatomy of the biliary tree

The biliary tree is divided into extrahepatic and intrahepatic bile ducts. The right and left hepatic duct, and common hepatic duct are collectively called hilar bile ducts. The intrahepatic bile ducts, proximal to the right of the left hepatic duct, are classified as the intrahepatic large and small bile ducts.[15] The former are visible grossly, and consist of the first to third branches of the right or left hepatic bile duct. The latter are recognizable microscopically, consist of septal and interlobular bile ducts, and are connected to bile ductules. The septal bile duct is surrounded by a fibrous wall and over 100 μm in external diameter. While the external diameter of the interlobular bile duct is under 100 μm. These two bile ducts are accompanied by hepatic arterial branches, while bile ductules are located at the periphery of portal tracts.

Classification of ICC

Classification of ICC based on combined gross and histological features

ICC is grossly classifiable into three types: mass-forming (MF); periductal infiltrating (PI); and intraductal growths (IG).[1,16,17] The MF type presents as a nodular or mass lesion in the hepatic parenchyma, and the carcinoma is gray to gray–white, firm and solid. The PI type shows spreading of the carcinoma along the portal tracts with biliary stricture of the involved bile ducts and dilatation of the peripheral bile ducts. The IG type presents as a polypoid or papillary tumor within the dilated bile duct lumen.

In this study, ICC were classified based on gross features and the cancerous involvement of the intrahepatic large bile ducts into two categories: peripheral and perihilar.

Peripheral ICC. ICC showing no involvement of intrahepatic bile large ducts are included in this type. ICC involving intrahepatic small bile ducts and those showing no evident involvement of the intrahepatic biliary tree are included as well. This type usually shows features of the MF type. Histologically, ICC are classifiable into adenocarcinomas and variants. The former are dividable into two types according to the size of tubules or acini of the carcinoma and their pattern of proliferation: bile duct type and bile ductular type.[18,19]

1 Bile duct adenocarcinoma. This is an invasive adenocarcinoma with a variable-sized tubular structure or lumen, acinar, trabecular and/or micropapillary structures, and also a cribriform formation and/or a cord-like pattern (Fig. 1). Some parts or cases show a large tubular or even microcystic adenocarcinoma with small cord-like, or micropapillary components in varying combinations. This tumor is usually divided into well, moderately, and poorly differentiated types according to cellular and structural atypia. The carcinoma cells invade the hepatic parenchyma by compressing the hepatocytes, infiltrating along the sinusoids, and replacing directly the adjoining hepatocytes. They also show portal venous or lymphatic invasion in portal tracts or fibrous septa, and perineural invasion in large portal tracts. Mucin is found in the acini, along the luminal sides, and in the cytoplasm of carcinoma cells. Fibrous stromal reaction and inflammatory reactions are variable.

2 Bile ductular adenocarcinoma. This type is characterized by the widespread growth of adenocarcinoma cells apparently replacing the hepatocytes within the hepatic lobules or regenerative nodules[18,19] (Fig. 2a,b). The carcinoma cells are usually small in comparison with those of bile duct ICC, and comparable to those in proliferative bile ductules, showing a small-cord like pattern. They are embedded in a fibrous stroma, and appear to recapitulate the canals of Hering or cholangioles. The acinus or lumen of these carcinomas is small or slit like. This type grows in a tubular, cord-like, anastomosing pattern, the so-called 'antler-like' pattern, and largely corresponds to cholangiolocellular carcinoma.[20] The shape or frame of pre-existing hepatic lobules or regenerative nodules is identifiable and the portal tracts or fibrous septa are regularly distributed within the tumor. The deposition of collagen fiber around or along the carcinoma cells is significant and carcinoma cells are squeezed here and there, with ghost-like features evident. The central areas of this type frequently show dense fibrosis and even scarring, while the peripheral regions show more actively proliferating carcinoma cells. Cellular atypia is usually mild and mucin production is absent or slight along the luminal side.

In addition, neoplastic structures mimicking the ductal plate malformation found in cases of Caroli's disease or congenital hepatic fibrosis[21] are also admixed (Fig. 2c). A central spot or bridge is evident microscopically. The tumors look benign, though they show infiltration growth.

3 Variants. The following rare variants of ICC were examined: anaplastic/undifferentiated carcinoma with occasional features of adenocarcinoma, adenosquamous/squamous carcinoma, mucinous carcinoma, signet-ring cell carcinoma, clear cell carcinoma, mucoepidermoid carcinoma, lymphoepithelioma-like carcinoma, neuroendocrine type, and sarcomatous type.

Perihilar ICC. ICC mainly involving intrahepatic large bile ducts or arising in intrahepatic large bile ducts are included in this type.

1 Periductal spreading or periductal MF type. A cancerous large bile duct shows a luminal spread of the carcinoma along the affected lumen with intraductal micropapillary carcinoma or *in situ* lesions, and at the same time, the carcinoma cells invade the duct wall and surrounding parenchyma. ICC arising from the large intrahepatic bile ducts show intraductal micropapillary carcinoma or *in situ* lesions along the biliary lumen. The affected bile ducts show thickened walls, stenotic, or obstructed.[1,10] As it is difficult to differentiate perihilar ICC showing periductal spreading from hilar cholangiocarcinoma (CC) at the time of surgical resection or autopsy, no such cases were included in this study.

2 Intraductal papillary neoplasm of the bile duct (IPNB). IPNB was proposed for the papillary neoplastic tumor in the intrahepatic large bile ducts. IPNB is not infrequently associated with invasion (invasive IPNB)[22,23] (Fig. 3). At the invasion site, a mucinous carcinoma or conventional tubular adenocarcinoma is found. About one third of IPNB secrete mucin in the duct lumen (mucin-secreting biliary tumor). IPNB includes biliary papilloma and papillomatosis. While IPNB is composed of a borderline lesion, *in situ* lesion and also invasive type, the latter two correspond to the IG type of ICC.[1,16,17] IPNB which seem to arise in the extrahepatic bile duct were not included in this study.

Patient selection and tissue preparation

Examinations of surgically resected cases

A total of 60 ICC cases (from 37 men and 23 women, ranging in age from 35 to 85 years with a mean of 73) from the Department of Human Pathology, Kanazawa University Graduate School of Medicine, Kanazawa and its affiliated hospitals from between 1998 and 2009 were used; 411 ICC cases (279 male and 132 female, aged 29 to 80 years with a mean of 59) from the Department of Pathology, University

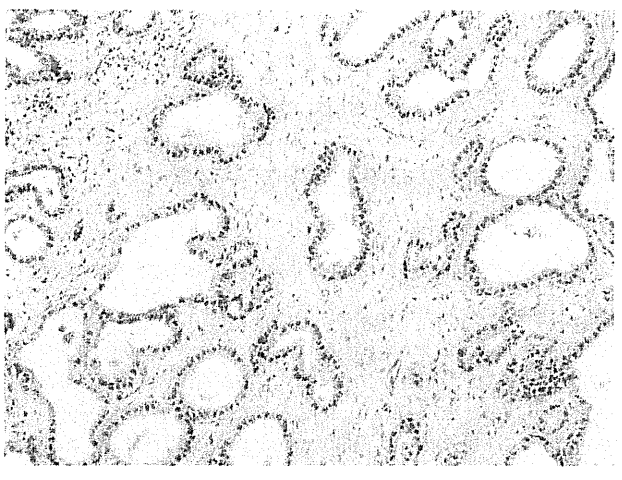

Figure 1 Bile duct type of intrahepatic cholangiocarcinoma. Well-differentiated tubular adenocarcinoma (HE stain).

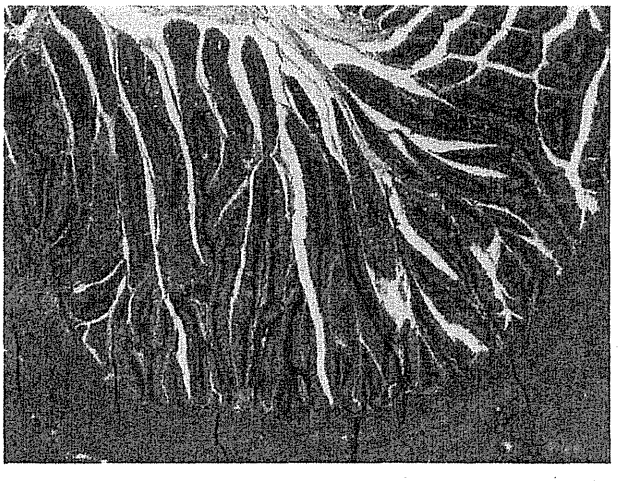

Figure 3 Intraductal papillary neoplasm of the bile duct. Carcinoma cells with a papillary configuration proliferating in the duct lumen (HE stain).

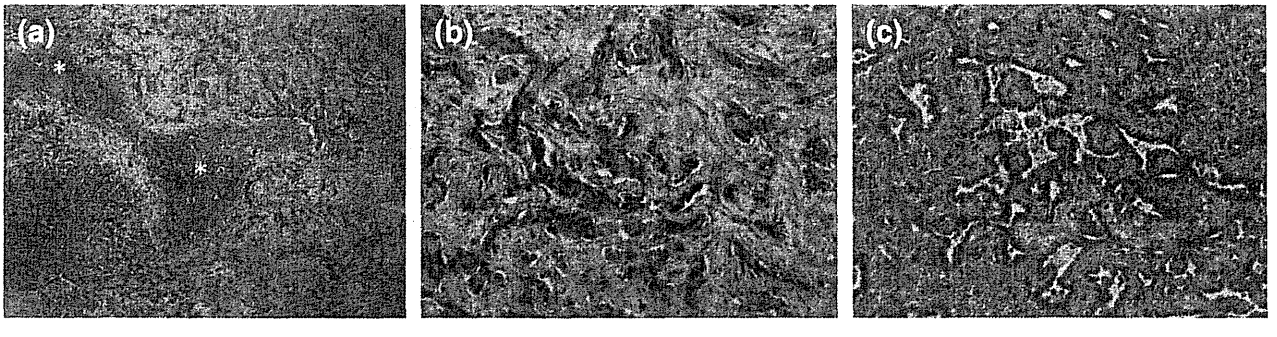

Figure 2 Bile ductular type of intrahepatic cholangiocarcinoma. (**a**) Carcinoma cells replacing hepatic parenchyma and portal tracts (*) are regularly distributed (HE). (**b**) Proliferating carcinoma cells resembling bile ductules. (**c**) Growth pattern resembling ductal plate malformation (HE).

of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea between 1998 and 2009 (Table 1) were studied. ICC arising in patients with chronic biliary diseases such as hepatolithiasis or PSC, biliary tract malformations including Caroli's disease, and combined hepatocellular CC were not included in the present study. While no liver flukes were found, the possibility of clonorchiasis or related biliary damage was not excluded. As shown in Table 1, in the Kanazawa University series (60 cases), 17 cases of ICC were associated with non-biliary CALD which included liver cirrhosis and advanced liver fibrosis with lobular disarray; 12 cases were related to HCV infection, two cases to non-alcoholic steatohepatitis (NASH), one case to HBV infection, and one case to chronic alcoholism, while the background in the remaining case was unclear. These cases were categorized as group A (chronic advanced, non-biliary liver diseases; non-biliary CALD). The remaining 43 patients showed an almost normal liver or non-specific reactive hepatitis (NSRH) but no bridging fibrosis or lobular disarray. These cases were categorized as group B (apparently normal livers or NSRH). NSRH, defined as mild portal inflammation or fibrosis with or without mild lobular inflammation, is known to develop as a secondary feature in liver tissue around the tumor or in a liver with gastrointestinal or systemic disease.[24] In the Asan Medical Center series (411 cases), 36 cases of ICC were associated with non-biliary CALD (group A); 26 cases were related to HBV infections, two cases to HCV infections, and two cases to chronic alcoholism, while the etiology was unclear in the remaining six cases. The other 375 patients showed an almost normal liver or NSRH but no bridging fibrosis or lobular disarray (group B).

Examinations of autopsy cases

All autopsied cases in the Department of Pathology, Kanazawa University Graduate School of Medicine were surveyed from 1988 to 2008 to evaluate the incidence of ICC associated with chronic liver disease. During this period, a total of 3400 autopsies were done. ICC including peripheral ICC and perihilar ICC arising in the intrahepatic large bile duct, and hepatocellular carcinoma (HCC) were examined. ICC were classified according to the background lesions: ICC arising in an apparently normal liver; ICC arising in chronic liver diseases such as chronic viral hepatitis and nonalcoholic steatohepatitis; and ICC arising in chronic chronic biliary diseases such as hepatolithiasis and PSC.

Table 1 Main features of intrahepatic cholangiocarcinoma cases examined in this study (surgically resected cases)

	Total	Group A	Group B
Kanazawa University	60 cases	17 cases	43 cases
Age (years)	35–85 (mean 68)	44–74 (mean 67)	35–85 (mean 69)
Sex (male : female)	37:23	9:8	28:15
Asan Medical Center	411 cases	36 cases	375 cases
Age (years)	29–80 (mean 59)	37–70 (mean 58)	29–80 (mean 60)
Sex (male : female)	279:132	29:7	250:125

Group A, chronic advanced, non-biliary liver diseases; Group B, apparently normal livers or non-specific reactive hepatitis.

Tissue preparation

All the autopsied and surgically resected specimens were fixed in 10% buffered formalin. Representative sections from these specimens were embedded in paraffin. Sections greater than 4 μm thick were cut from each paraffin block and stained with HE, Azan-Mallory, reticulin and PAS after diastase digestion for histological observation.

Statistics

Intergroup comparisons were made by the χ^2 test or Fisher's exact test and these were used to compare frequencies of categorical variables; $P < 0.01$ was regarded as statistically significant.

RESULTS

Comparison of subtypes of ICC in surgically resected cases

Comparison of subtypes of ICC arising in groups A and B

Subtypes of ICC were found in both group A and group B. The majority of ICC were of the bile duct type in both groups, 36 of 53 cases (67.9%) in group A and 268 of 418 cases (64.1%) in group B (Table 2). Interestingly, most were well- to moderately-differentiated adenocarcinomas (27 of 36 cases (75%) in group A and 236 of 268 cases (88.1%) in group B). The poorly-differentiated type was infrequent in both groups. It was found that in group A the bile ductular type was frequent (12 of 53 cases, 22.6%) compared with group B (35 of 418 cases, 8.4%; $P < 0.001$). As for the background of ductular ICC as a whole, 12 of 47 cases were associated with group A (25.5%), while 35 of 47 cases were associated with group B (74.5%). Variants of ICC were rare in group A (three cases (5.7%), all undifferentiated ICC) and also rare in group B (21 cases(5%); 12 cases of undifferentiated ICC, six cases of squamous/adenosquamous cell carcinoma, two cases of sarcomatous ICC, and one case of mucinous carcinoma) (Table 2). In contrast, IPNB was rather frequent in group B (94 of 418 cases, 22.5%) in comparison with group A (2 of 53 cases, 3.8%; $P < 0.001$).

Because the bile duct type of ICC and variants of ICC were found similarly in groups A and B, it seems plausible that bile

Table 2 Comparison of intrahepatic cholangiocarcinoma arising in chronic advanced, non-biliary, and biliary diseases (group A) and apparently normal livers or non-specific reactive hepatitis (group B)

	Group A (53 cases)	Group B (418 cases)	Statistics*
Bile duct type	36 cases (67.9%)	268 cases (64.1%)	$P = 0.585$
Well-differentiated	9 cases	102 cases	
Moderately-differentiated	18 cases	134 cases	
Poorly-differentiated	9 cases	32 cases	
Bile ductular type	12 cases (22.6%)	35 cases (8.4%)	$P < 0.001$
Variants	3 cases (5.7%)	21 cases (5%)	$P = 0.843$
IPNB	2 cases (3.8%)	94 cases (22.5%)	$P < 0.001$

*The χ^2 test; IPNB, intraductal papillary neoplasm of bile duct.

Table 3 Comparison of intrahepatic biliary neoplasm between Kanazawa University and Asan Medical Center; intrahepatic cholangiocarcinoma arising in chronic advanced, non-biliary, liver diseases; and intrahepatic cholangiocarcinoma arising in apparently normal livers or nonspecific reactive hepatitis

Comparison of intrahepatic biliary neoplasm between Kanazawa University and Asan Medical Center			
	Kanazawa University (60 cases)	Asan Medical Center (411 cases)	Statistics*
Bile duct type	50 cases (83.3%)	254 cases (61.8%)	$P < 0.001$
Well	28 cases	83 cases	
Moderate	16 cases	136 cases	
Poor	6 cases	35 cases	
Bile ductular type	6 cases (10%)	41 cases (10%)	$P = 0.995$
Variants	3 cases (5%)	21 cases (5.1%)	$P = 0.971$
IPNB	1 cases (1.7%)	95 cases (23.1%)	$P < 0.001$
Intrahepatic cholangiocarcinoma arising in chronic advanced, non-biliary, liver diseases			
	Kanazawa University (17 cases)	Asan Medical Center (36 cases)	Statistics*
Bile duct type	13 cases (76.5%)	23 cases (63.9%)	$P = 0.360$
Well	6 cases	3 cases	
Moderate	4 cases	14 cases	
Poor	3 cases	6 cases	
Bile ductular type	3 cases (17.6%)	9 cases (25%)	$P = 0.550$
Variants	1 (cases 5.9)	2 cases (5.6%)	$P = 0.962$
IPNB	0 cases (0%)	2 cases (5.6%)	$P = 0.322$
Intrahepatic cholangiocarcinoma arising in apparently normal livers or nonspecific reactive hepatitis			
	Kanazawa University (43 cases)	Asan Medical Center (375 cases)	Statistics*
Bile duct type	37 cases (86.0%)	231 cases (61.6%)	$P < 0.002$
Well	22 cases	80 cases	
Moderate	12 cases	122 cases	
Poor	3 cases	29 cases	
Bile ductular type	3 cases (7%)	32 cases (8.5%)	$P = 0.727$
Variants	2 cases (4.7%)	19 cases (5.1%)	$P = 0.906$
IPNB	1 case (2.3%)	93 cases (24.8%)	$P < 0.001$

*The χ^2 test; IPNB, intraductal papillary neoplasm of bile duct.

ductular type ICC has a tendency to develop in group A while IPNB is unlikely to develop in this group.

Comparison of proportions of subtypes of ICC between Kanazawa University (Japan) and Asan Medical Center (South Korea)

As shown in Table 3, IPNB was frequent in the Asan Medical Center series (95 of 411 cases (23.1%)) in comparison with the Kanazawa University series (one of 60 cases (1.7%)) ($P < 0.001$). The difference was significant in group B ($P < 0.001$), but not group A (Table 3). Interestingly, in the Asan series, IPNB was frequent in group B (24.8% of 375 cases) but rare in group A (5.6% of 36 cases) ($P < 0.01$), and in the Kanazawa series, rare in group A (0%) and group B (2.3%), suggesting that IPNB is likely to develop in group B in Seoul. The bile duct type accounted for the majority of cases in both institutions (83.3% of 60