Int J Clin Exp Pathol 2015;8(7):8284-8290
www.ijcep.com /ISSN:1936-2625/IJCEP0009452

Original Article

Pathological characteristics of intraductal polypoid neoplasms of bile ducts in Thailand

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Received April 21, 2015; Accepted June 26, 2015; Epub July 1, 2015; Published July 15, 2015

Abstract: Intraductal papillary or tubular neoplasms of the bile duct have recently been proposed as one of the pre-invasive lesions of cholangiocarcinoma. Herein, a total of 50 cases of intraluminal polypoid neoplasms of the bile ducts experienced in Khon Kaen University Hospital in Thailand were pathologically examined. These cases presumably had a history of infection of *Opisthorchis viverrini*. These neoplasms were histologically composed of high-grade intraepithelial neoplasm showing a tubular and/papillary pattern without invasion (20 cases), and with minimal and considerable invasion (15 and 15 cases, respectively). They were histologically classifiable into papillary type (10 cases), tubular type (20 cases) and papillotubular type (20 cases), and were phenotypically classifiable into gastric (17 cases), intestinal (17 cases) and pancreatobiliary types (16 cases). It was found that cases of papillary type and gastric or intestinal phenotype were less invasive, while those of tubular or papillotubular type and pancreatobiliary phenotype were more invasive. In conclusion, intraductal polypoid neoplasms in Thailand were well-differentiated papillary and/or tubular neoplasms including those with no or minimal invasion, and histological and phenotypic subclassifications seem to be useful for evaluation of the aggressive pathological behaviors of these neoplasms.

Keywords: Biliary tree, intraductal papillary neoplasm, intraductal cholangiocarcinoma, papillary cholangiocarcinoma, phenotype, liver fluke infection

Introduction

Cholangiocarcinoma (CC) is a highly malignant neoplasm and relatively infrequent cancer in Western countries, while it is not infrequently found in East Asia. Risk factors for CC in East Asian countries have been reported to differ from those in Western countries [1, 2]. In the latter, primary sclerosing cholangitis is the most important risk factor, with a relative risk of developing CC of 10-30% in comparison with the general population. In the former, particularly in northeast parts of Thailand and Korea, many studies suggest a close association between liver fluke infection and the development of CC.

Liver fluke infection by *Opisthorchis viverrini*, *Opisthorchis felineus* and *Clonorchis sinensis* is one of the major public health problems in East Asia and Eastern Europe. At present, more than 600 million people are at risk of infection with these trematodes [1-5]. Liver flukes chronically habitat the bile duct lumen, leading to chronic inflammation, proliferation of bile duct lining epithelium and peribiliary glands, dysplasia of these epithelia and, eventually, the development of CC. The possible mechanisms of carcinogenesis induced by liver flukes include nitric oxide formation, chronic irritation, intrinsic nitrosation and activation of drug-metabolizing enzymes [3-5]. However, the precise molecular mechanisms behind cholangiocarcinogenesis in liver fluke infection remain poorly understood.

Recently, the 2010 WHO classification of biliary tract tumors proposed a novel subtype of pre-invasive neoplastic biliary lesion, intraductal papillary neoplasm of bile duct (IPNB) [1, 6, 7]. Intraductal tubular or intraductal papillotubular neoplasm is also proposed as a pre-invasive

Table 1. Antibodies employed for immunohistochemistry

Molecule	Clone	Source	Dilution	Antigen retrieval
HepPar-1	OCH1E5	DAKO	1:100	MW
CDX2	EPR2764Y	Nichirei	Prediluted	MW (pH 9.0)
MUC1	DF3	TFB	1:100	-
MUC2	Ccp58	Novocastra	1:100	MW
MUC5AC	CLH2	Novocastra	1:200	MW
MUC6	CLH5	Novocastra	1:200	MW
CK7	OV-TL 12/30	DAKO	1:50	MW
CK20	Ks20.8	DAKO	1:50	MW
SOX9	3F11	Abnova	1:500	MW
Lgr5	EPR3065Y	LSBio	1:500	Autoclave
PDX1	PAb	Santa Cruz Biotechnology	1:500	MW
Ezrin	PAb	Cell Signaling	1:500	MW
S100P	EPR6143	Abcam	1:100	MW
γ-H2AX	EP854(2)Y	Novus Biologicals	1:50	MW

Abbreviations: CK, cytokeratin; MW, microwaving in 10 nmol/L citrate buffer (pH 6.0) for 20 minutes; PAb, polyclonal antibody.

neoplasm of the biliary tree [6, 8-10]. These conditions are finally followed by invasive CC. Recent studies have shown that these subtypes are not infrequently found in the liver infected by *Clonorchis sinensis* in Korea [11, 12]. CCs are known to arise frequently in the liver infected by *Opisthorchis viverrini* [3, 4]. Pathological features of these CCs are well known and about one-third of CCs are reported to show intraductal papillary lesion [5]. However, there have been no detailed pathological studies on these subtypes of biliary tumors in the liver infected by *Opisthorchis viverrini*.

Herein, we pathologically and immunohistochemically examined a total of 50 surgically resected cases of intraductal polypoid neoplasm of the bile duct in Thailand, focusing on papillary and/or tubular histological components and their phenotypes.

Materials and methods

Cases and tissue preparation

A total of 50 surgically resected cases of biliary neoplasm showing intraluminal polypoid lesions of the bile duct were obtained from Khon Kaen University Hospital in Thailand. They were obtained from 35 males and 15 females (median age 63, range 46-77 years). These cases had a history of eating raw fish, presumably indicative of a history of liver fluke infection by *Opisthorchis viverrini*. All operations for these cases were regarded as curative. At least three parts were available from the affected bile duct, and they were fixed in 10% formalin and embedded in paraffin. More than 20 serial thin sections were cut from each paraffin block, and were processed for histologic observations by routine staining including hematoxylin-eosin staining and also for immunohistochemistry.

Histopathological evaluation

Bile duct lesions of these 50 cases were examined histopathologically. The amount of papillary or tubular component was evaluated and each case was assigned to one of three types according to the following criteria: (i) papillary type, if the lesion was composed of > 75% papillary or villous growth; (ii) tubular type, if the lesion was composed of > 75% tubular growth; and (iii) papillotubular type, if two respective components constituted more than 25% of the lesion. Furthermore, these cases were subdivided into four phenotypes (gastric, intestinal, pancreatobiliary and oncocytic types) with the aid of immunostaining of MUC1, MUC2, MUC5AC, MUC6, CDX2, cytokeratin 7 (CK7) and CK20 according to the WHO classification of pancreatic and biliary neoplasms [1, 7]. When there were more than two phenotypes within the neoplasms, the predominant type was employed as the phenotype for the case.

Immunohistochemistry

The sections were used for immunohistochemical staining of MUC1, MUC2, MUC5AC, MUC6, CDX2, CK7 and CK20 as an aid to phenotyping of the tumors. The expression of several molecules related to carcinogenesis and the progression of pancreatobiliary carcinoma, such as S100P, SOX9, Lgr5, PDX1, Ezrin and γ-H2AX, was also examined for characterization of the

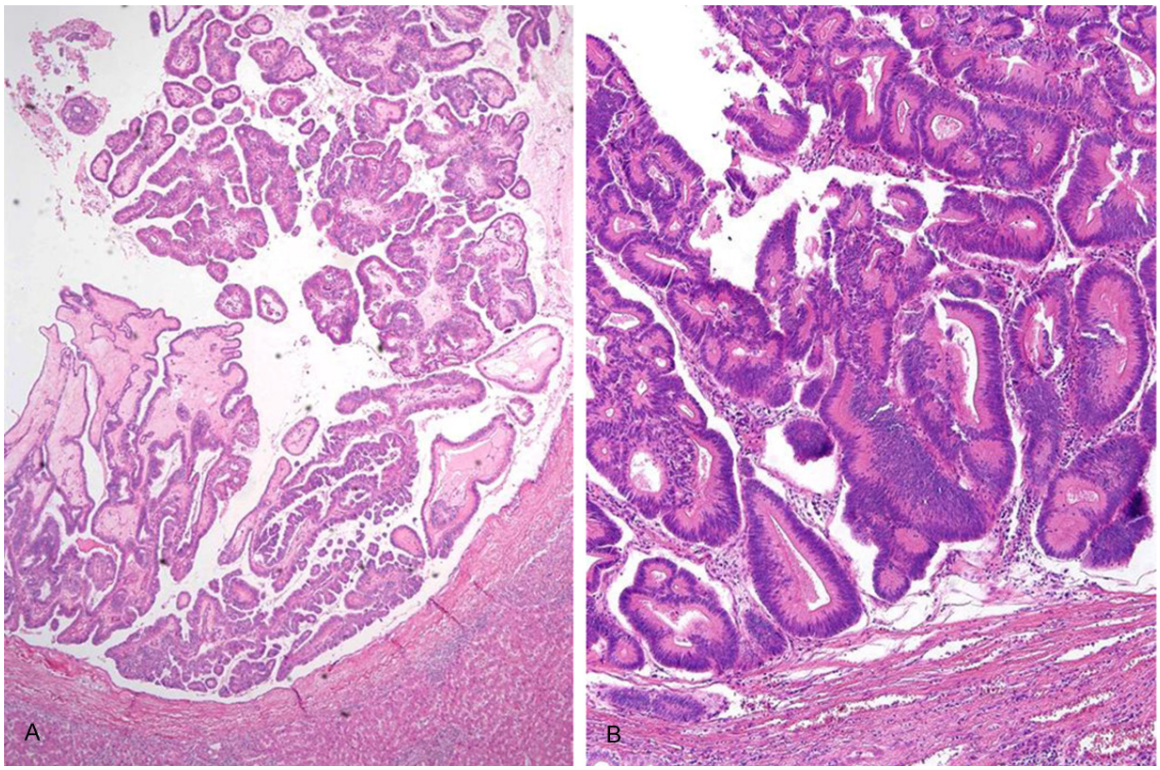

Figure 1. Intraductal polypoid neoplasm. Papillary type and pancreatobiliary phenotype (A). Tubular type and intestinal phenotype (B). Hematoxylin-eosin staining. Original magnifications: (A) × 100; (B) × 150.

tumors in relation to their histologic subtypes [13-18]. The primary antibodies and their sources, optimal dilution and antigen retrieval methods are shown in **Table 1**. After blocking of the endogenous peroxidase, the sections were incubated overnight at 4°C with each of the primary antibodies. They were then treated with secondary antibodies conjugated to a peroxidase-labeled polymer using the HISTOFINE system (Nichirei, Tokyo, Japan). Color development was performed using 3,3'-diaminobenzidine tetrahydrochloride. The sections were counterstained with hematoxylin. Negative controls were produced by substituting the primary antibody with nonimmunized serum, which resulted in no signal detection.

The immunohistochemical expression of S100P, SOX9, Lgr5, PDX1, Ezrin and γ-H2AX was semiquantitatively graded in consideration of the proportion of positive cells in each lesion as follows: 0, negative or less than 5% of tumor cells were positive; 1+ (mild), 5% to 20% of tumor cells were positive; 2+ (moderate), 20% to 50% of tumor cells were positive; 3+ (marked) more than 50% of tumor cells were positive.

Statistical analysis

Statistical significance was determined using the Mann-Whitney *U*-test and Chi-square for independence test. A *P* value of less than 0.05 was accepted as the level of statistical significance. Statistical analysis was performed using the JMP 10.0 software package (SAS Institute, Inc., Cary, NC).

Results

Pathological features of intraductal polypoid tumor of the bile duct in Thailand

The intraductal neoplasms were mainly located at the intrahepatic large bile duct in 21 cases, at the perihilar bile duct in 28 cases and at the distal bile duct in the remaining one case. Histologically, these neoplasms were composed of papillary and/or tubular, high-grade intraepithelial neoplasm without invasion (preinvasive carcinoma, 20 cases) and invasion (invasive carcinoma, 30 cases). Mucus hypersecretion was not found in these cases. The amount of fibrous stroma of these tumors was

Table 2A. Histologic subtypes of intraductal polypoid lesions and invasion in intraductal polypoid neoplasm of the bile duct

Histologic type	No invasion	Invasion (minimal, considerable)
Papillary type (n = 10)	8	2 (1, 1)
Tubular type (n = 20)	6	14 (12, 2)
Papillotubular type (n = 20)	6	14 (2, 12)

n, number of cases. $P < 0.01$.

Table 2B. Phenotypes of intraductal polypoid lesions and invasion in intraductal polypoid neoplasm of the bile duct

Phenotype	No invasion	Invasion (minimal, considerable)
Gastric type (n = 17)	10	7 (5, 2)
Intestinal type (n = 17)	9	8 (4, 4)
Pancreatobiliary type (n = 16)	1	15 (6, 9)

n, number of cases. $P < 0.01$.

Table 2C. Correlation between histolotic subtypes and phenotypes in intraductal polypoid neoplasm of the bile duct

Histologic type	Gastric type	Intestinal type	Pancreatobiliary type
Papillary type (n = 10)	4	4	2
Tubular type (n = 20)	5	6	9
Papillotubular type (n = 20)	8	7	5

n, number of cases.

small and narrow in all cases. Among the cases with invasion, 15 cases showed invasion limited to the duct wall, while the remaining cases showed invasion into the periductal tissue including liver parenchyma. Invasive parts showed tubular adenocarcinoma, and mucinous carcinoma was not found. As for the histologic subtypes, 10 cases were of papillary type (**Figure 1A**), 20 cases of tubular type (**Figure 1B**) and the remaining 20 cases of papillotubular type. As for the phenotypes, 17 cases were of intestinal type, 17 cases of gastric type and the remaining 16 cases of pancreatobiliary type. There were no cases of oncocytic type.

Histological subtype and phenotype

As shown in **Table 2A**, 8 of 10 cases of papillary type showed no invasion, while 14 of 20 cases of tubular type and 14 of 20 cases of papillotubular type showed invasion (papillary vs. tubular or papillotubular, both $P < 0.01$). Invasive tubular type frequently showed considerable invasion in comparison with invasive papillotubular type. As shown in **Table 2B**, 10 of 17 cases of gastric type and 8 of 16 cases of intestinal type showed no invasion, while 15 of 16 cases of pancreatobiliary type showed invasion (pancreatobiliary vs. gastric or intestinal, $P < 0.01$). As for the relationship between histologic subtype and phenotype, there was no significant correlation between them (**Table 2C**).

Immunohistochemical analysis

Lgr5 and Ezrin were detected in the cytoplasm or membranous staining pattern (**Figure 2A**), while Sox9, PDX1 and γ-H2AX showed nuclear staining. S100P showed cytoplasmic and nuclear expression (**Figure 2B**). **Figure 3** shows a comparison of the degree of immunohistochemical expression of S100P, SOX9, Lgr5, PDX1, Ezrin and γ-H2AX and histologic subtypes of intraductal polypoid lesions of the bile ducts. There were no significant differences between the expression status of these markers and the histological subtype of the lesions.

Discussion

The findings obtained in this study can be summarized as follows: i) intraductal polypoid neoplasms of the bile duct in Thailand were composed of high-grade intraepithelial neoplasm without invasion (preinvasive carcinoma) and with invasion (invasive carcinoma); ii) they were histologically classified into papillary, tubular and papillotubular types, and phenotypically into gastric, intestinal and pancreatobiliary types; and iii) intraductal polypoid lesions with papillary type and gastric or intestinal type were less invasive, while those with tubular or papillotubular type and pancreatobiliary type were more invasive. Taken together, the subclassification of histologic subtype and phenotype of intraductal polypoid neoplasms seems to be useful for evaluation of their pathological aggressiveness.

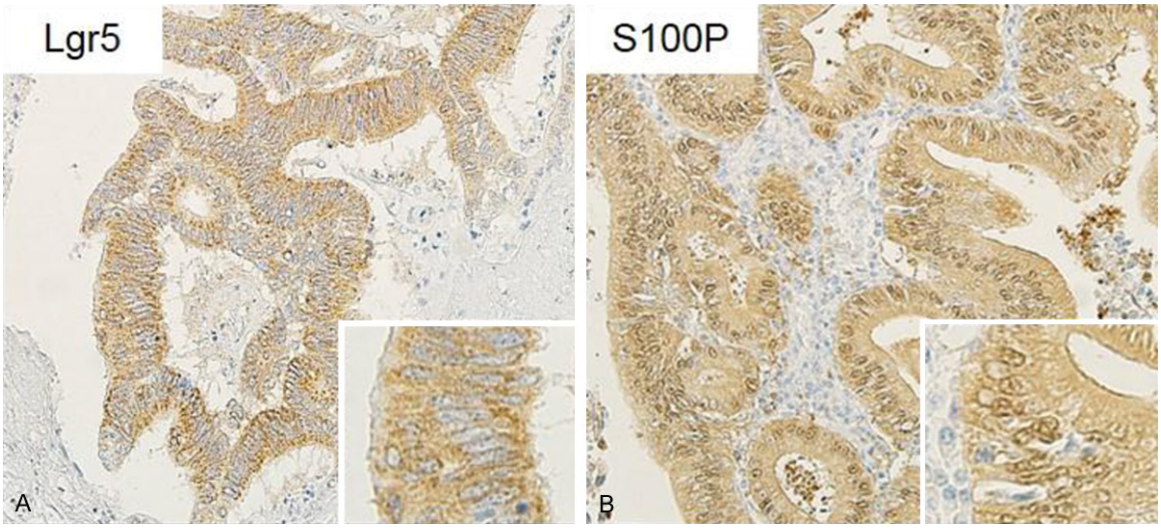

Figure 2. Immunohistochemical analysis of intraductal polypoid neoplasm. Expression of Lgr5 in the cytoplasm (A). Expression of S100P in the cytoplasm and nuclei (B). Original magnifications: (A, B) × 200 (inset, higher magnification).

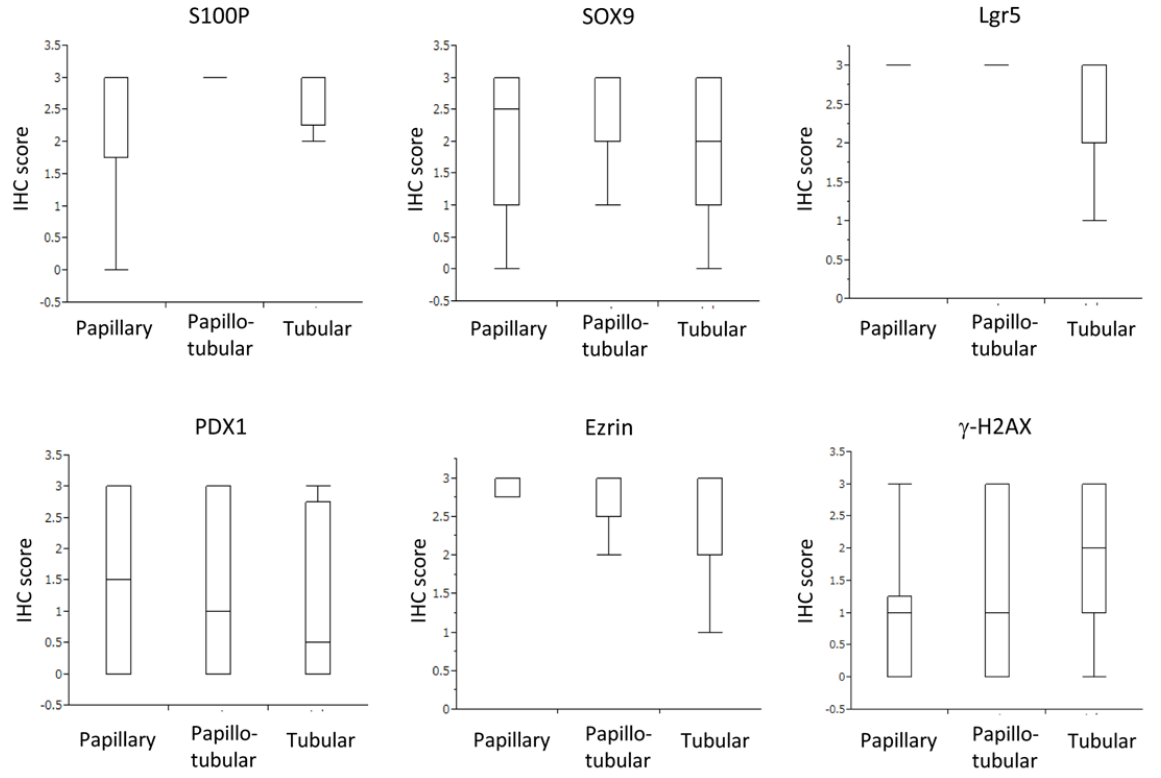

Figure 3. Comparison of the immunohistochemical results and histologic subtype. Semiquantitative analysis of the immunohistochemistry (IHC) was performed as described in the Materials and methods. There was no significant difference in the histologic subtype and the immunohistochemical expression of S100P, SOX9, Lgr5, PDX1, Ezrin and γ-H2AX.

It was found that intraductal polypoid neoplasms of the bile duct in Thailand showed high-grade intraepithelial neoplasia with papillary and tubular structures. Interestingly, 20 of

50 cases showed no invasion. While the remaining 30 cases showed invasion, 15 cases showed only minimal invasion. Fibrous stroma was small and narrow. In the hepatobiliary neoplasms related to liver fluke infection, recent studies from Korea showed that IPNB was not infrequent in liver infected by *Clonorchis sinensis*, in which about half of these tumors were non-invasive IPNB and borderline or low/intermediate cases were also included [11, 12]. The intraductal polypoid neoplasms in this series in Thailand included considerable tubular components, and also showed pathologic and invasive characters similar to IPNB associated with *Clonorchis sinensis* infection.

Histologically, these neoplasms were classifiable into tubular type (20 cases), papillary type (10 cases) and papillotubular type (20 cases). In relation to invasion, papillary type was usually non-invasive, while tubular and papillotubular types were frequently associated with invasion. However, in relation to the degree of immunohistochemical expression of S100P, SOX9, Lgr5, PDX1, Ezrin and γ-H2AX, which were reported to be related to carcinogenesis and the progression of pancreatobiliary carcinoma [13-18], these histological subtypes were not related, suggesting that these intraductal polypoid neoplasms might undergo similar carcinogenetic processes irrespective of the proportion of tubular or papillary components.

Phenotypically, the intraductal polypoid neoplasms in this series were classifiable into gastric (17 cases), intestinal (17 cases) and pancreatobiliary types (16 cases). There were no cases of oncocytic type. As for the phenotype of IPNB related to *Clonorchis sinensis* infection, Jang et al. reported that pancreatobiliary type was predominant, while Jung et al. reported that intestinal and gastric types were predominant [11, 12]. This difference in Korean cases may be related to the method of phenotyping. In the intraductal polypoid lesions in Thailand, gastric, intestinal and pancreatobiliary types were similarly found. Although there was no correlation between histological subtype and phenotype in this series, pancreatobiliary type showed more invasive character than gastric or intestinal type.

Recently, intraductal papillary or tubular neoplasms of the bile duct have been proposed as early or pre-invasive lesions of CC [1, 7]. This study showed that intraductal polypoid neoplasm of the bile duct showed no invasion, minimal invasion or considerable invasion at the time of surgical resection. Therefore, it is important in clinical practice to identify the markers including histologic features in order to predict aggressive behavior or invasion. In the cases of intraductal polypoid neoplasm of the bile duct related to *Opisthorchis viverrini* infection, it was found that cases of papillary type and gastric or intestinal phenotype were less invasive, while those of tubular or papillotubular type and pancreatobiliary phenotype were more invasive. Detection of such histologic elements would predict the aggressiveness of these tumors.

In conclusion, intraductal polypoid neoplasm of the bile duct in Thailand was well-differentiated papillary and/or tubular neoplasm, and the subclassification based on histologic subtype and phenotype seems to be useful for the evaluation of their pathological behavior.

Disclosure of conflict of interest

None.

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