



Development of intraductal papillary neoplasm of the bile duct in patients with occupational cholangiocarcinoma

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Abstract

This study aimed to investigate the clinicopathological findings of intraductal papillary neoplasm of the bile duct (IPNB) in patients with occupational cholangiocarcinoma caused by exposure to 1,2-dichloropropane and/or dichloromethane to elucidate the development of IPNB to cholangiocarcinoma. The clinicopathological findings of 17 IPNB lesions according to the size (1.5–20 mm in diameter) and their comparison between type 1 ($n = 9$) and type 2 IPNBs ($n = 8$) were investigated. An IPNB of <5 mm in size was classified as micro IPNB ($n = 7$), while those ≥ 5 mm in size were classified as ordinary IPNB ($n = 10$). Both types 1 and 2 IPNBs were observed in micro IPNB, and their proportions were not different between micro and ordinary IPNBs. The clinicopathological characteristics of types 1 and 2 IPNBs were fundamentally similar to those previously reported. IPNB with invasive carcinoma was only found in ordinary IPNB although the proportions of low-grade and high-grade epithelium were not different between micro IPNB and ordinary IPNB. All IPNB exhibited γ H2AX and S100P, indicating the occurrence of DNA injury and malignant transformation in micro and ordinary IPNBs. These results indicate that the carcinogens may induce micro IPNB with types 1 or 2 at the initiation and then develop ordinary IPNB with types 1 or 2, subsequently, progressing to IPNB with invasive carcinoma in patients with occupational cholangiocarcinoma.

Keywords Intraductal papillary neoplasm of the bile duct · Occupational cholangiocarcinoma · Mucin production · Malignant transformation · Micro IPNB

Introduction

Intraductal papillary neoplasm of the bile duct (IPNB) is a premalignant lesion of intra- and extrahepatic cholangiocarcinoma and is characterized as a grossly visible lesion with intraductal papillary or villous growth of biliary epithelium [1]. IPNBs have been detected in patients with hepatolithiasis, liver fluke infection, primary sclerosing cholangitis, or occupational cholangiocarcinoma caused by long-term

exposure to high 1,2-dichloropropane (1,2-DCP) and/or dichloromethane (DCM) concentrations [2–5].

Recently, a new pathological diagnostic criterion for IPNB was proposed, wherein lesions were classified into types 1 and 2, according to their similarity to intraductal papillary mucinous neoplasm of the pancreas [1, 6]. A collaborative study by the Japan Biliary Association and Korean Association of Hepato-Biliary-Pancreatic Surgery reported the differences in clinicopathological characteristics and prognosis between types 1 and 2 IPNBs [7]. Moreover, Aoki et al. reported that IPNBs consist of two distinct types (types 1 and 2) associated with different clinicopathological features and molecular phenotypes [8]. Recently, Luvira [9] proposed an IPNB progression model, indicating that all IPNBs start from a “micro-papillary type.” However, the clinicopathological findings of such micro-papillary type IPNB and the development through the initiation of IPNBs to invasive carcinoma remained uncertain [1–3, 6–8].

We had previously reported that IPNBs often develop in patients with occupational cholangiocarcinoma [4, 5, 10–12]. Kinoshita et al. reported that 11 of 16 patients with

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occupational cholangiocarcinoma had IPNBs with or without invasive carcinoma, with some of the patients having multiple IPNBs [10]. This series revealed that the IPNBs included some intraductal growing lesions of <5 mm in diameter although the size of IPNB is usually 5–20 mm [1]. The carcinogenic process of occupational cholangiocarcinoma involves chronic bile duct injury with DNA damage in almost all large bile ducts by DCP and/or DCM exposure, along with precancerous lesion development, such as biliary intraepithelial neoplasia (BilIN) and IPNB, and eventually invasive cholangiocarcinoma [7, 8, 10, 12, 13]. Moreover, the whole-exome analysis revealed that occupational cholangiocarcinoma and precancerous lesions have a high mutational burden and a unique trinucleotide mutation signature of GpCpT to GpTpY [14, 15].

The current study investigated the clinicopathological findings of consecutive sizes of IPNB, including lesions of <5 mm in diameter, and compared pathological findings between types 1 and 2 IPNBs to clear the development through the initiation of IPNB to IPNB with invasive carcinoma using the pathological specimens in patients with occupational cholangiocarcinoma that has the established etiologic and carcinogenic background.

Materials and methods

Patients

This study included seven patients with occupational cholangiocarcinoma and IPNB in the intrahepatic bile ducts (Table 1) because most IPNBs developed in the intrahepatic bile ducts in occupational cholangiocarcinoma. Among the seven patients, six had occupational cholangiocarcinoma confirmed by the Ministry of Health, Labour and Welfare, Japan. Another patient was assumed to have occupational cholangiocarcinoma although without the desire to register it as an occupational disease with the Ministry because of his long-term high 1,2-DCP and DCM concentration exposure [16]. All patients (average age: 40 years; age range: 31–47 years; all males) were former or present workers of a printing company in Osaka who were exposed to high 1,2-DCP and/or DCM concentrations over a range of 6 years and 1 month to 18 years. A total of 17 IPNB lesions were pathologically evaluated in the seven patients (patients 1, 2, 3, and 6 had one IPNB lesion and patients 3, 5, and 7 had 4, 7, and 2 IPNB lesions, respectively, Table 1). Of the 17 IPNB lesions, 5 were detected on diagnostic imaging with a size of >10 mm in diameter.

Each lesion was identified using the patient-lesion number format, such that lesion 1 in patient 1 was named lesion 1-1 (Table 1).

The study was approved by the ethics committee of Osaka City University (current Osaka Metropolitan University; Nos. 2368, 2840, 3455, and 4063). All subjects provided written informed consent.

Pathological examination

Pathological examination was performed using surgical specimens from six patients and autopsy specimens from one patient. The multicentric carcinogenesis (multicentric occurrence) of IPNB, BilIN, and cholangiocarcinoma is an important and typical occupational cholangiocarcinoma characteristic [4, 5, 10, 11, 13, 15]. We evaluated the clinicopathological characteristics, including the independent presence of cholangiocarcinoma and the pre- or early cancerous lesions, such as BilIN and IPNB, according to the World Health Organization classifications for intrahepatic cholangiocarcinoma [1, 4, 5, 11]. Observed lesions, including the main tumor(s), BilIN, and IPNB on surgical specimens, were cross-referenced with the anatomical charts of the bile duct. The detailed macroscopic and microscopic pathological findings of the surgical specimens were mapped to evaluate the correlation between the anatomical charts of the large bile duct using diagnostic imaging, such as CT, magnetic resonance cholangiopancreatography, and endoscopic retrograde cholangiopancreatography, and pathological findings.

The main tumor was a mass-forming type intrahepatic cholangiocarcinoma (patient 4), a distal cholangiocarcinoma (patient 6), and IPNB with invasive carcinoma in five other patients. Three patients (patients 3, 5, and 7) had 4, 7, and 2 independent IPNB lesions, respectively.

IPNBs, which are characterized by the independent presence of intraductal papillary or villous biliary neoplasms covering delicate fibrovascular stalks, can be pathologically classified into two types. The first type (type 1 IPNB) is histologically similar to intraductal papillary mucinous neoplasms of the pancreas and typically develops in the intrahepatic bile ducts, while the second type (type 2 IPNB) has a more complex histological architecture with irregular papillary branching bile ducts and is frequently associated with invasive carcinomas [1, 6].

IPNBs were classified into two groups according to the size of the intraductal polypoid or papillary structures from their base. IPNBs of <5 mm in size were classified as “micro IPNB” and IPNBs of ≥ 5 mm in size were classified as “ordinary IPNB” because IPNB is a grossly visible lesion whose size is usually ≥ 5 mm [1].

IPNBs were classified with low-grade and high-grade intraepithelial neoplasias and invasive carcinoma components based on the cytological atypia and architectural features. The severer grade was adopted. Epithelial cellular types were further classified into subtypes (i.e., intestinal,

Table 1 Characteristics of patients with intraductal papillary neoplasm of the bile duct (IPNB)

Patient no.	Age	Organic solvents	Operative methods	Main tumor	IPNB					Grade	Diagnostic imaging
					Lesion no.	Location	Size (mm)	Type	Subtype		
1	40	DCP, DCM	Extended left bisectonectomy	IPNB with invasive carcinoma of the common hepatic duct	1-1	B4	20	2	Pancreatobiliary	High	Detected
2	39	DCP	Extended left bisectonectomy, resection of extrahepatic duct	IPNB with invasive carcinoma of the intrahepatic duct	2-1	B4	15	2	Pancreatobiliary	High	Detected
3	39	DCP	Left bisectonectomy, S7 segmentectomy	IPNB with invasive carcinoma of the intrahepatic duct	3-1	B7	13	2	Oncocytic	High	Detected
4	31	DCP	Right bisectonectomy, resection of extrahepatic duct	Mass-forming type intrahepatic cholangiocarcinoma	4-1	B7	10	2	Oncocytic	High	Not detected
5	34	DCP	Extended left bisectonectomy, resection of extrahepatic duct*	IPNB with invasive carcinoma of the intrahepatic duct	5-1	B4	16	2	Oncocytic	High	Detected
6	42	DCP, DCM	Chemotherapy (GEM + CDDP), stent	Distal cholangiocarcinoma	5-2	B3	6	1	Gastric	Low	Not detected
					5-3	B2	4	2	Pancreatobiliary	High	Not detected
					5-4	B4	4	1	Pancreatobiliary	High	Not detected
					5-5	B4	2	1	Gastric	Low	Not detected
					5-6	B4	2	2	Pancreatobiliary	High	Not detected
					5-7	B3	1.5	2	Pancreatobiliary	High	Not detected
					6-1	B2	13	1	Gastric	Low	Detected
7	47	DCP, DCM	Extended left bisectonectomy	IPNB with invasive carcinoma of the intrahepatic duct	7-1	B4	15	1	Pancreatobiliary	High	Detected
					7-2	B3	4	1	Gastric	Low	Not detected

gastric, pancreatobiliary, and oncocytic) according to the most prevalent morphological pattern [1, 2].

Mucin staining was performed using double mucin stain with a periodic acid-Shiff stain after diastase-digestion and alcian blue (pH 2.5) (d-PAS/AB) as previously reported [17]. The amount of mucin production was classified as high, low, or none, based on d-PAS/AB positive mucin on the cell surface and cytoplasm [17].

Immunohistochemistry

Immunohistochemical staining was performed using primary antibodies (Supplemental Table 1). Antigen retrieval was performed by microwaving in 10 nmol/L citrate buffer (pH 6.0). Sections were incubated in protein block solution (Dako-Cytomation, Glostrup, Denmark) and then incubated overnight at 4 °C with each of the primary antibodies after blocking endogenous peroxidase. Thereafter, they were 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, and sections were lightly counter-stained with hematoxylin.

The expression of mucin core proteins (MUC1, MUC2, MUC5AC, and MUC6) was immunohistochemically assessed using a previously described method [18]. Cytoplasmic and/or luminal immunostaining of MUC1 and cytoplasmic immunostaining of MUC2, MUC5AC, and MUC6

were evaluated. Antibodies against γ H2AX and S100P were used to evaluate DNA injury locations and neoplastic changes, respectively. IPNBs were observed in the fields at 200 \times magnification, and the nuclear expression of γ H2AX and nuclear and cytoplasmic expressions of S100P were assessed [11, 13].

Statistical analysis

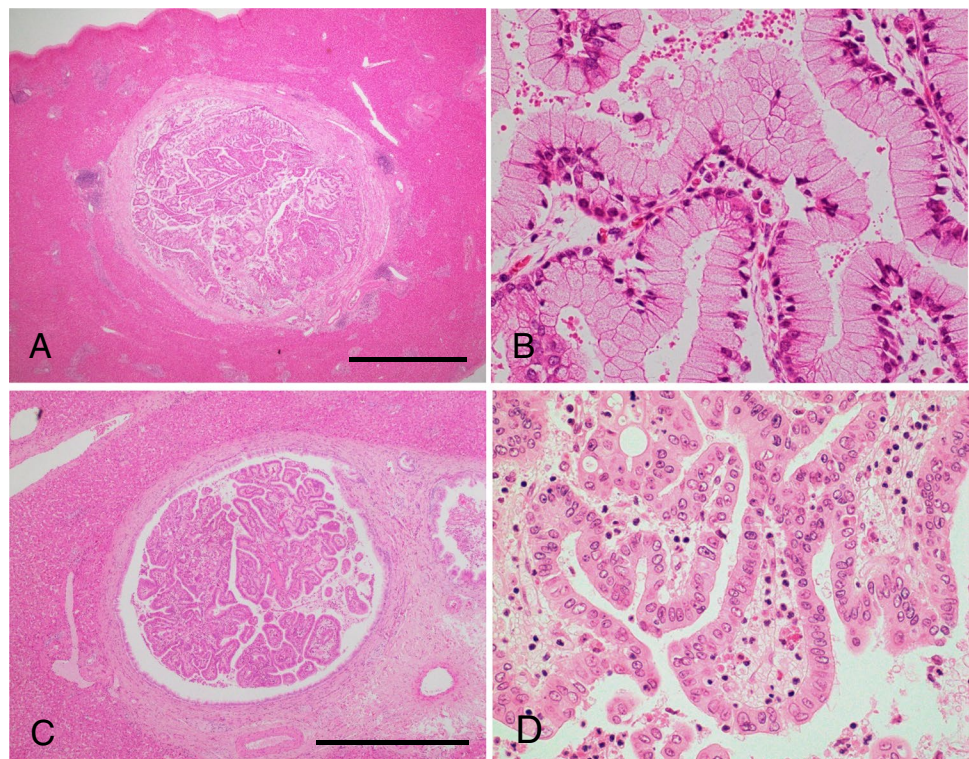
Student's *t* test was used to compare continuous variables, while Fisher's exact test was used for categorical variables. All statistical analyses were performed using EZR, with a *p* value of <0.05, indicating statistical significance.

Results

Pathological findings of micro IPNB

The clinicopathological findings of the 17 IPNBs ranged from 1.5 to 20 mm in diameter (Table 1). Of the 17 IPNBs, 7 were classified as micro IPNB (<5 mm in diameter), and 10 were classified as ordinary IPNB (\geq 5 mm in diameter). The typical pathologic findings of micro IPNB are shown in Figs. 1 and 2. Figure 1A, B shows a micro IPNB (lesion 7-2, 4 mm in diameter, type 1, gastric type), and Fig. 1C, D shows a micro IPNB (lesion 3-4, 1.5 mm, type 1, pancreatobiliary type). In three IPNB lesions classified into oncocytic type (lesions, 3-1,

Fig. 1 Histology of micro intraductal papillary neoplasms of the bile duct (IPNBs). **A** (H-E, scale bar 2 mm, \times 20) and **B** (\times 400) shows a micro IPNB (lesion 7-2), and **C** (H-E, scale bar 1 mm, \times 40) and **D** (\times 400) show a micro IPNB (lesion 3-4). Lesion 7-2 is classified as type 1 and gastric type. Lesion 3-4 is classified as type 1 and pancreaticobiliary type



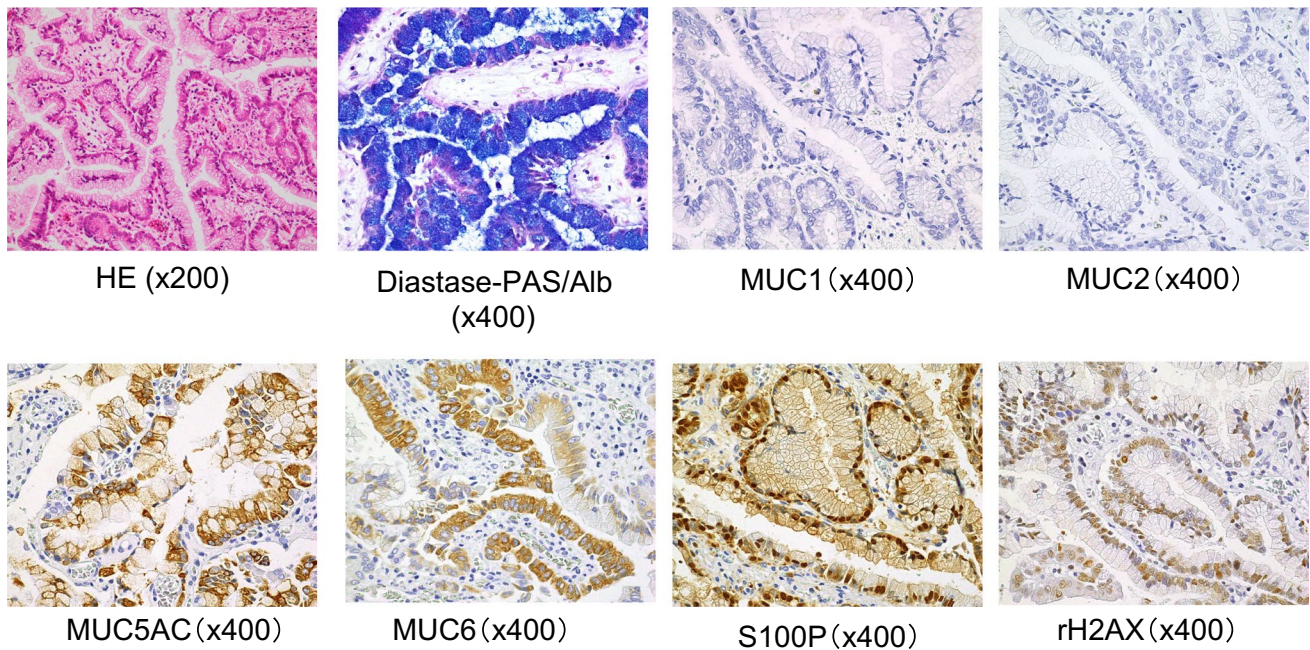


Fig. 2 Immunohistochemical finding of IPNB (lesion 7-2). **A**, HE; **B**, Diastase-PAS/Alb; **C**, MUC1; **D**, MUC2; **E**, MUC5AC; **F**, MUC6; **G**, S100P; **H**, rH2AX. Lesion 7-2 has high mucin production (surface

and cytoplasm). The lesion was positive for MUC5AC and MUC6 whereas negative for MUC1 nor MUC2. The lesion exhibited γ H2AX and S100P expression

4-1, and 5-1), the IPNB composed of mainly oncocytic type and coexisted with pancreatobiliary type. No IPNB lesions composed of oncocytic type alone were found.

Supplemental Figure 1 shows multiple lesions of micro IPNB in patient 5, including A: lesion 5-1, 16 mm, ordinary IPNB, type 2; B: the lesion 5-1 composed of mainly oncocytic type (left side) and admixed pancreatobiliary type (right side); C: lesion 5-1, invasive area of ordinary IPNB; D: lesion 5-2, ordinary IPNB, 6 mm, type 1, gastric type; E: lesion 5-3, 4 mm, micro IPNB, type 1, pancreatobiliary type; F: lesion 5-4, 4 mm, micro IPNB, type 2, pancreatobiliary type; G: lesion 5-5, 2 mm, micro IPNB, type 1, gastric type; H: lesion 5-6, 2 mm, micro IPNB, type 2, pancreatobiliary type; I: lesion 5-7, 1.5 mm, micro IPNB, type 2, pancreatobiliary type.

BillIN lesion was detected at various sites of the bile duct in all seven patients, as previously reported [4, 11, 12, 16].

Figure 2 shows the representative immunohistochemical findings (lesion 7-2). A high mucin production (surface and cytoplasm) was observed. The lesion was positive for MUC5AC and MUC6 whereas negative for MUC1 nor MUC2. The lesion exhibited γ H2AX and S100P expressions.

Clinicopathological findings of IPNBs according to their size

Table 2 shows the clinicopathological findings of IPNB according to their size. Diagnostic imaging detected six

lesions of ten ordinary IPNBs, whereas no lesions were detected in micro IPNB ($p = 0.0345$).

Types 1 and 2 IPNBs were found in 4 and 3 lesions in micro IPNB and 5 and 5 lesions in ordinary IPNB, respectively. Types 1 and type 2 IPNB revealed no different proportions between micro and ordinary IPNBs, and both types 1 and 2 IPNBs developed in micro and ordinary IPNBs. The proportions of low-grade and high-grade epithelium were not different between micro and ordinary IPNB. IPNB with invasive carcinoma was not detected in micro IPNB, while 5 of 10 ordinary IPNBs were classified as IPNB with invasive carcinoma. The proportion of IPNB with invasive carcinoma was significantly higher in ordinary IPNB than in micro IPNB. Lymph node metastasis was detected in one ordinary IPNB. The subtypes (pancreatobiliary, gastric, oncocytic, etc.) were not different between micro and ordinary IPNBs. The proportion of mucin production was not different between micro and ordinary IPNBs. The proportions of MUC1, MUC2, MUC5AC, and MUC6 positivity were not different between micro and ordinary IPNBs. All IPNBs exhibited γ H2AX and S100P expression, indicating the occurrence of DNA injury and malignant transformation in micro and ordinary IPNBs.

Clinicopathological findings of types 1 and 2 IPNBs

Of the 17 IPNBs, 9 and 8 lesions were classified as types 1 and 2, respectively (Table 3).

Table 2 Clinicopathological findings in micro IPNB and ordinary IPNB

Findings	Micro IPNB (<5 mm)	Ordinary IPNB (≥5 mm)	
	<i>n</i> = 7	<i>n</i> = 10	<i>p</i>
Detection on diagnostic imaging	0	6	0.0345
Type 1, type 2	4, 3	5, 5	>0.999
Grade (Low, High)	2, 5	3, 7	>0.999
Invasive carcinoma	0	5	0.0441
Lymph node metastasis	0	1	>0.999
Subtypes			
Pancreatobiliary	5	5	0.279
Gastric	2	2	
Oncocytic	0	3	
Intestinal	0	0	
Mucin production			
Surface (high, low, or none)	2, 5	5, 5	0.622
Cytoplasmic (high, low, or none)	3, 4	5, 5	>0.999
Positive immunohistochemical staining			
MUC1	4	4	0.637
MUC2	0	2	0.485
MUC5AC	5	6	>0.999
MUC6	1	3	0.603
γH2AX	7	10	>0.999
S100P	7	10	>0.999

Table 3 Clinicopathological findings in type 1 IPNB and type 2 IPNB

Findings	IPNB		<i>p</i>
	Type 1 (<i>n</i> = 9)	Type 2 (<i>n</i> = 8)	
Detection of diagnostic imaging	2	4	0.335
Size (mm, range, mean ± SD)	1.5–15.0, 6.2 ± 4.7	1.5–20.0, 10.2 ± 7.0	0.178
Grade (low, high)	5, 4	0, 8	0.0294
Invasive carcinoma	0	5	0.009
Lymph node metastasis	0	1	0.471
Subtypes			
Pancreatobiliary	5	5	0.0307
Gastric	4	0	
Oncocytic	0	3	
Intestinal	0	0	
Mucin production			
Surface (high, low, or none)	6, 3	1, 7	0.0498
Cytoplasmic (high, low, or none)	7, 2	1, 7	0.0152
Positive immunohistochemical staining			
MUC1	1	8	0.0406
MUC2	1	2	0.535
MUC5AC	7	4	>0.999
MUC6	4	0	0.580
γH2AX	9	8	>0.999
S100P	9	8	>0.999

Of the nine type 1 IPNB lesions, 5 and 4 were classified as low-grade epithelium and high-grade epithelium, respectively, whereas all eight type 2 IPNB lesions were classified as high-grade epithelium. The proportions of high-grade IPNB were significantly higher in type 2 IPNB than in type 1 IPNB ($p = 0.0294$). The IPNB with invasive carcinoma was found in five of the eight type 2 IPNBs, with significantly higher proportions in type 2 IPNB than in type 1 IPNB ($p = 0.0090$). The lymph node metastasis was detected in one type 2 IPNB lesion.

Of the nine type 1 IPNB lesions, 5 and 4 were classified as pancreatobiliary and gastric, respectively (Table 3). Of the eight type 2 IPNB lesions, 5 and 3 lesions were classified as pancreatobiliary and oncocytic, respectively.

Significantly more type 1 IPNBs had high mucin production (surface and cytoplasm) than type 2 IPNBs ($p = 0.0498$ and $p = 0.0152$, respectively). Moreover, significantly more type 2 IPNBs exhibited MUC1 positivity than type 1 IPNBs ($p = 0.0406$). The proportions of MUC2, MUC5AC, and MUC6 positivity were not different between types 1 and 2 IPNBs. All IPNBs exhibited γH2AX and S100P expression.

Discussion

IPNB is a premalignant or early stage cholangiocarcinoma and has a heterogenous pathologic character, with various etiologies. Many studies have reported the differences

in clinicopathologic features, molecular phenotypes, and prognosis [1, 6, 7]. Luvira [9] proposed a model of IPNB progression, indicating that all IPNBs start from a “micro-papillary type.” However, the clinicopathologic findings of “micro-papillary type” IPNB and the development of the IPNB remain unclear because such small lesions could not be detected on diagnostic imaging, as in the present study. Therefore, the current study investigated the clinicopathologic characteristics of IPNBs, including micro IPNBs, in patients with occupational cholangiocarcinoma, considering the high incidence of IPNBs among patients with occupational cholangiocarcinoma (e.g., 11 of 16 patients with occupational cholangiocarcinoma had IPNBs) and the established etiologic and carcinogenic background of IPNBs.

This study classified 17 IPNB lesions into 7 micro IPNBs and ten ordinary IPNBs. The proportion of types 1 and 2, grade (low and high), subtypes (pancreatobiliary, gastric, oncolytic, intestinal), mucin production, and positivity for MUC1, MUC2, MUC5AC, and MUC6 were not different between micro and ordinary IPNBs although the proportion of IPNB with invasive carcinoma was significantly higher in ordinary IPNB than in micro IPNB. All IPNBs exhibited γ H2AX and S100P expression. Previous studies in patients with occupational cholangiocarcinoma revealed a positive γ H2AX for chronic bile duct injury, pre- or early cancerous lesion (IPNB and biliary intraepithelial neoplasia; BilIN), and invasive carcinoma, and S100P was positive for pre- or early cancerous lesion (IPNB and biliary intraepithelial neoplasia; BilIN) and invasive carcinoma [11, 13]. The results in this study indicate that not only ordinary IPNB but also micro IPNB has already transformed with DNA injury. Mimaki et al. revealed that micro IPNB shared the trinucleotide mutational signature of GpCpY to GpTpY and a high mutation burden, which are characteristic findings of occupational cholangiocarcinoma [15]. Additionally, the multicentric occurrence of cholangiocarcinoma is a characteristic of occupational cholangiocarcinoma because pre- and early cancerous lesions, including IPNB, develop based on the high potential of malignant transformation at various sites of the bile ducts caused by DCP and/or DCM. This series detected multiple IPNBs, including micro IPNBs, in three patients (patients 3, 5, and 7). Invasive carcinoma components and lymph node metastasis develop during the progression from micro IPNB to ordinary IPNB.

Recently, IPNBs are classified into types 1 and 2 and according to the differences in clinicopathological and molecular biological findings between types 1 and 2 IPNBs. Type 1 IPNBs often present with low-grade intraepithelial neoplasia and infrequently exhibit invasion. Conversely, type 2 IPNBs mainly exhibit high-grade intraepithelial neoplasia and are often associated with invasive carcinoma [1, 2]. Kubota et al. revealed that 9.7% and 40.2% of type 1 IPNBs were classified as low/intermediate-grade and high-grade,

respectively, whereas most type 2 IPNBs were classified as those with invasive carcinoma. Additionally, Aoki et al. revealed that type 1 IPNBs were classified as low- and high-grade, whereas type 2 IPNBs was predominantly classified as those with invasive carcinoma [4]. The current study classified five of the nine types 1 IPNBs as low-grade intraepithelial neoplasm, whereas all type 2 IPNBs were classified as high-grade intraepithelial neoplasm. IPNB with invasive carcinoma was observed in only type 2 IPNBs. Therefore, the proportion of IPNB classified as high-grade intraepithelial neoplasm and that of IPNB with invasive carcinoma were significantly higher in type 2 IPNB than in type 1 IPNB. Almost all IPNBs exhibited a varying degree of mucin production [17]. Accordingly, excessive mucin production was generally observed in type 1 IPNB but rarely in type 2 IPNBs [1], similar to the current study. Zen et al. revealed that increased MUC1 expression was associated with carcinogenic pathways in hepatolithiasis [5]. The present study revealed that significantly more type 2 IPNBs (with invasive carcinoma) exhibited MUC1 positivity than type 1 IPNBs although MUC1 positivity was not different between micro IPNB and ordinary IPNB. Therefore, MUC1 positivity was closely related to the type of IPNB (types 1 or 2) rather than the size of IPNB and to the IPNB progression with invasive carcinoma.

The present study revealed that micro IPNB included both types 1 and 2 IPNBs. Additionally, both types 1 and 2 IPNBs were positive for γ H2AX and S100P and had high tumor mutation burden, as shown in the previous study [15], indicating that DNA injury with mutation and malignant transformation occurred at the early stage of IPNB (micro IPNB). Moreover, we revealed the consecutive size of IPNBs (micro IPNB and ordinary IPNB with or without invasive carcinoma) in three patients (patients 3, 5, and 7) and the overall series in this study. These findings indicate that similar carcinogen (DCP and/or DCM) induces micro IPNB with types 1 or 2 IPNBs at the initiation and then develops ordinary IPNB with types 1 or 2, subsequently, progressing to IPNB with invasive carcinoma. This study revealed different subtype distributions (pancreatobiliary, gastric, oncolytic, and intestinal) and mucin production patterns between types 1 and 2 IPNBs. However, the relationship in the development and progression between types 1 and 2 remained unclear. Therefore, further examination of the relationship between types 1 and 2 IPNBs is necessary.

A recent study by Singi et al. [19] reported that *PRKACA* or *PRKACB*-related fusion genes were detected in 3 IPNB lesions with oncocytic type. The gene analysis of the two IPNB lesions with oncocytic prevalent type (lesions 3-1 and lesion 5-1) in the previous study [14] showed that *PRKACA* or *PRKACB*-related fusion genes were not detected although mutations of *PRKACG*, *PRKAG3*, and *PRKAR2B* were identified in lesion 5-1. The analysis also identified the mutations

of *SMAD4*, *AXIN1*, *PIK3CA*, and *ATM* in lesion 3-1 and those of *TP53*, *KRAS*, *CDKN2A*, *ARID1A*, and *AXIN1* in lesion 5-1, as commonly mutated genes in biliary tract cancer. In this study, the IPNB lesions with oncocytic type prevalent IPNB lesions coexisted with pancreatobiliary type. The coexistence of pancreatobiliary type IPNB lesion and invasive IPNB in this study might be related to the discrepancy.

The BilIN is a premalignant lesion of cholangiocarcinoma and is often detected at various sites of the bile ducts in patients with occupational cholangiocarcinoma. This study detected BilIN in all seven patients. However, the differences in the mechanism of the development between IPNB, including micro IPNB, and BilIN are unclear. Nakanuma et al. revealed that the practical differentiation of high-grade BilINs with micro-papillary patterns from intracystic papillary neoplasm and IPNB has not yet been discussed in detail [20].

One limitation of the current study was the small number of IPNBs, considering the limited number of patients with occupational cholangiocarcinoma. Additionally, elucidating the characteristics of IPNB at very early stage is necessary. Moreover, genetic analysis is necessary to obtain definitive conclusions although genetically analyzing all IPNBs is often difficult because of the small size of the lesions (especially, micro IPNB).

In conclusion, the current study in patients with occupational cholangiocarcinoma revealed that IPNB included micro IPNB (<5 mm in diameter) with malignant transformation and that the clinicopathological characteristics of types 1 and 2 IPNBs were fundamentally similar to those previously reported. Similar carcinogens (DCP and/or DCM) may induce such IPNB with types 1 or 2 at the initiation and then develop ordinary IPNB with types 1 or 2, and, subsequently, progressing to IPNB with invasive carcinoma.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00428-023-03499-5>.

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Author contributions Study design: SK, MK, and YS designed the study. Acquisition of data: S. Tanaka, MK, HS, and TI. Pathological aspect of the study: MK and YS. Data analysis: SK, MK, and YS. Manuscript drafted by SK and YS. All authors reviewed the manuscript.

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Declarations

Ethics approval The study was approved by the ethics committee of Osaka City University (current Osaka Metropolitan University).

Conflict of interest The authors declare no competing interests.

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