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Original Article

Intraductal papillary neoplasm of bile duct is associated with a unique intraepithelial spreading neoplasm

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Abstract: Cholangiocarcinoma is not infrequently associated with intraepithelial spread of neoplastic biliary cells in the biliary mucosa around the main carcinoma which is called here "intraepithelial spreading neoplasm (IESN)". Herein, the pathological features and significance of IESN of intraductal papillary neoplasm of bile duct (IPNB) were examined by using 52 cases of IPNB with reference to those of IPNB itself. As a control, 23 cases of nodular sclerosing cholangiocarcinoma (NS-CCA) with IESN were used. It was found that IPNB were constantly associated with IESN, and IESN and IPNB showed the similar biological and pathological features in individual cases. Interestingly, invasive lesion(s) were found at IESN in 22 of and also at IPNB in 32 of 52 cases, and invasion was tended to be found at both lesions in the same cases. IESN of IPNB was classifiable histologically into flat (52 cases), and lower and higher micropapillary types (52 and 43 cases, respectively), while IESN(s) in NS-CCA were only classifiable into flat (23 cases) and low micropapillary (12 cases) types. Intestinal, gastric, pancreatobiliary (PB) and oncocytic phenotypes were found similarly in IPNB and IESN of IPNB, while PB type was predominant in NS-CCA and in LSIN of NS-CCA. These findings suggest that IPNB and IESN compose a unique intraepithelial neoplasm of the biliary tree with imminent potential of invasion along any place.

Keywords: Intraductal papillary neoplasm, intraepithelial spread, biliary tree, invasion, cholangiocarcinoma

Introduction

In the intrahepatic and extrahepatic bile ducts, two types of intraepithelial lesions have been proposed as a premalignant or pre-invasive lesion: biliary intraepithelial neoplasm (BilIN) and intraductal papillary neoplasm of bile duct (IPNB) [1]. While BilIN is a flat and microscopically recognizable lesion and usually followed by or associated with nodular sclerosing cholangiocarcinoma (NS-CCA) including flat/nodular type of CCA of extrahepatic bile duct and periductal infiltrating type of CCA of intrahepatic large bile duct, while IPNB is characterized by grossly visible papillary tumor in the dilated bile ducts and usually followed by intraductal papillary growth type of CCA (IPNB with an associated invasive carcinoma) [1, 2].

IPNB is characteristically composed of well-differentiated epithelial neoplasm covering fine fibrovascular cores and presents four subtypes (intestinal, gastric, pancreatobiliary (PB) and oncocytic type) [1-6]. IPNB is categorized into three grades: low, intermediate and high grade intraepithelial neoplasm (*in situ* carcinoma). High grade IPNB is associated with or eventually followed by invasive adenocarcinoma (CCA) [1, 2, 5, 7]. IPNB is reported to resemble intraductal papillary mucinous neoplasm of pancreas (IPMN) [8-13] which is also known to present three grades of intraepithelial neoplasm and to be followed by invasive adenocarcinoma (IPMN with an associated invasive carcinoma), though the proportion of high grade intraepithelial neoplasm and the incidence of invasion are reportedly higher in IPNB than in IPMN [5, 11-13].

There have been several reports about the intraepithelial spread of neoplastic cells in the surrounding biliary mucosa around hilar and extraheaptic CCAs [3, 14, 15]. While this lesion has been called by various names such as mucosal extension of carcinoma, this lesion is here called "intraepithelial spreading neoplasm (IESN)". Sakamoto et al. [14] firstly reported

that IESN was seen not infrequently in hilar CCA, and since then, there have been several studies on its prognostic significance [15, 16]. Nakanishi et al. [15] reported that intraductal papillary carcinoma of hilar bile duct was frequently associated with IESN in comparison with other gross types of hilar CCA. IPNB is also reported to be frequently associated with IESN in the surrounding biliary mucosa [4-7, 13]. While there have been many clinicopathological and molecular studies on IPNB itself [1, 2, 4, 6, 7, 10, 17, 18], the pathological features and significance of LSIN associated with IPNB remain to be clarified. Interestingly, intraepitheilal spread of neoplastic cells is also known in the surrounding pancreatic ducts of IPMN, and invasive carcinoma can be identifiable at the bottom of IPMN and more frequently at the surrounding intraepithelial neoplasm [10].

Invasive ductal carcinoma of the pancreas is reported to invade backward into and extend along the ductal system (cancerization), morphologically mimicking pancreatic intraepithelial neoplasia (PanIN) of high grade or even IPMN [19]. Basturk et al. pointed out the difficulty to differentiate high grade PanIN from cancerization, and they proposed the term “intraductal spread of invasive carcinoma” for this lesion [19]. This may be the case in NS-CCA, and IESN in NS-CCA can include BilIN, particularly high grade BilIN, and also possibly cancerization of CCA, implicating that IESN in NC-CCA and possibly in IPNB may be heterogeneous.

In spite of this limitation, this is the first study to evaluate the pathological features and significance of IESN in the biliary mucosa around IPNB by using a total of 52 cases of surgically resected IPNB.

Materials and methods

Definition of IPNB, IESN and NS-CCA

IPNB

IPNB is defined by dilated bile ducts filled with a grossly visible noninvasive papillary biliary neoplasm covering delicate fibrovascular stalks. Covering cells are well-differentiated cuboidal to columnar neoplastic epithelial cells [1]. Only IPNB higher than 5 mm in its height from the basal lamina of the bile duct was included in this series. Moderately to poorly differentiated papillary and papillotubular adenocarcinoma or tubular neoplasm containing a few papillary components appearing as a grossly polypoid lesions within the dilated ducts were also not included in this study.

NS-CCA

This type is a common type of CCA [1, 2] and presents the diffuse and/or nodular thickening of the affected bile ducts with infiltration of tubular or cord-like adneocarcinoma with desmoplastic reaction. The luminal surface showed micropapillary or flat adenocarcinoma histologically but not grossly visible papillary carcinoma.

IESN

This lesion was grossly granular or rough or unremarkable on the biliary mucosa and histologically intraepithelial continuous, well-differentiated cuboidal to columnar neoplastic epithelia on the biliary mucosa [2, 14-16]. IESN was continuous with grossly visible papillary neoplastic lesions of IPNB itself or from NS-CCA. Abrupt transition or front formation of IESN against the background non-neoplastic biliary epithelia was not infrequently found.

Patient selection and tissue preparation

A total of 52 cases of IPNB were chosen from 326 surgically resected cases with a diagnosis of bile duct tumor at Shizuoka Cancer Center, Shizuoka, Japan (2002-2013). These cases were used for the comparative study between IPNB and IPMN [13]. The study protocol was approved by the institutional review board of Shizuoka Cancer Center. As a control, 23 cases of perihiar and distal NS-CCA showing IESN were chosen from the file of CCA in Shizuoka Cancer Center (2010-2014).

All of these tumors with enough proximal and distal bile ducts were available for the following examinations. About 20 tissue blocks including the main IPNB tumor (range; from 10 to 50 blocks) were sampled in individual cases for pathologic diagnosis. These blocks were fixed in 10% neutral buffered formalin, embedded in paraffin and processed routinely for histological observation. More than 20 serial thin sections were cut from two tissue blocks containing representative main tumor of IPNB and sur-

Table 1. Antibodies employed for immunohistochemistry

Target molecules	Antibody	Source	Dilution	Antigen retrieval
CDX2	EPR2764Y*	Nichirei	RTU	AC (pH 6)
MUC1	DF3*	TFB	1:100	-
MUC2	Ccp58*	Novocastra	1:100	AC
MUC5AC	CLH2*	Novocastra	1:200	AC
MUC6	CLH5*	Novocastra	1:200	AC
CK7	OV-TL 12/30*	DAKO	1:50	AC
CK20	Ks20.8*	DAKO	1:50	AC
CD10	56C6*	DAKO	1:100	AC

Abbreviation: AC, autoclaving in 10 nmol/L citrate buffer (pH 6.0) for 100 minutes; *, monoclonal antibody.

rounding bile ducts for the immunohistochemistry.

Subtypes and grades of IPNB, NS-CCA and LSIN

Four subtypes (intestinal, gastric, PB and oncocytic type) were examined in the main lesions of IPNB and NS-CCA and also in LSIN. Subtypes of IPNB were classified in consideration of histological characteristic of IPNB and IPMN in WHO 2010 classification of tumors of digestive system with a help of immunostaining of MUC1, MUC2, MUC5AC, MUC6, CDX2, CK7, CK20 and CD10 [1, 10]. In NS-CCA and IESN, histologically, gastric type was composed of foveolar pattern and pyloric gland pattern in a various combination, and intestinal type was composed of villous pattern and goblet cell pattern in a various combination. The PB type is tubular and micropapillary neoplasms composed of simple epithelia and the oncocytic type shows acidphilic oncocytic cells. Immunohistochemically, MUC5AC was used as a gastric foveolar marker, while MUC6 as a gastric pyloric gland marker. MUC2, CDX2 and CK20 were used as a marker of intestinal differentiation. CD10 is known to be expressed on brush border of intestinal epithelium and is used as an intestinal marker. CK7 and MUC1 were used to detect biliary differentiation. In IESN, particularly flat or pseudopapillary type, expression of immunohistochemical markers was evaluated more importantly in addition to histological features. When more than two subtypes were identified in an IPNB or NS-CCA or IENB, the predominant type was regarded as the subtype of the individual lesion or case. In control bile ducts, MUC1 is focally expressed on the apical membrane of the bile duct epithelia and peribiliary glands. MUC2, MUC5AC, CDX2, and CK20 are not expressed in bile duct epithelia. MUC6 and CD10 are variably expressed in peribiliary glands but not in the epithelial cells lining the bile ducts. CK7 is extensively expressed in the biliary epithelial cells and peribiliary glands.

The grade of IPNB was also done according to WHO 2010 classification (low/intermediate grade, high grade, and high grade with an associated invasive carcinoma) [1]. When only high grade or low/intermediate neoplasia was found in IESN, such case was classified as high grade or low/intermediate grade LSIN, respectively. When both lesions were found, such case was regarded as high grade.

Invasion from IENB and IPNB into bile duct wall

When tubular or cord-like adenocarcinomas in the ductal wall and periductal tissue were found unifocally beneath single IPNB lesion and/or single IESN lesion which were separate enough and when there were findings suggestive of direct invasion from the neoplastic lining epithelia of IPNB lesion and/or single IENB lesions into subepithelial carcinoma in the duct wall, such carcinoma lesion was regarded as "invasion" of carcinoma from individual IPNB lesion and/or IESN lesion, respectively. When such carcinoma cells were found beneath several separate IESN lesions and when there were findings suggestive of direct invasion from the neoplastic lining epithelia of IESN lesions, they were regarded as separate direction invasions from these IESNs. When such carcinoma cells in the duct wall beneath IPNB and IESN which were separate, were continuous or not clearly separated, it was not considered that invasion occurred from IESN independently from IPNB.

According to this presumption, IPNB and IESN were carefully scrutinized for evidence of invasion into the ductal wall. Depth of invasion was assessed into T1 and T2 [20]: the former was characterized by invasion limited to the duct wall and the latter by invasion beyond the ductal wall [20]. Intraepithelial involvement of carcinoma cells into the peribiliary glands was not regarded as invasion.

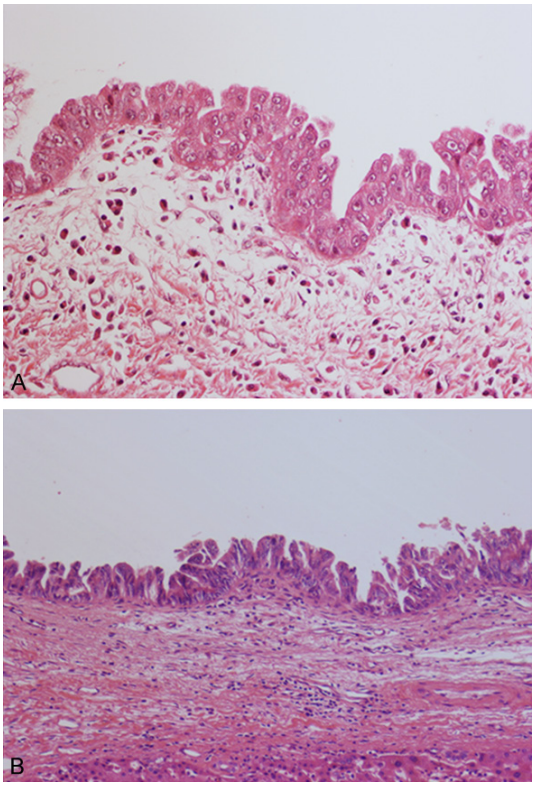

Figure 1. Intraepithelial spreading neoplasm of flat type. A: Flat type of oncocytic type. H&E, ×250 (original magnification). B: Flat type presenting with pseudopapillary pattern of pancreatobiliary type. H&E, ×200 (original magnification).

Immunohistochemistry

Immunohistochemical staining of MUC1, MUC2, MUC5AC, MUC6, CDX2, CK7, CK20 and CD10 was performed using formalin-fixed, paraffin-embedded tissue sections of surgically resected specimen for only evaluation of subtyping of IPNB, IESN and NS-CCA in this study. The primary antibodies and their sources, optimal dilution, and antigen retrieval methods are shown in **Table 1**. In brief, after pretreatment for antigen retrieval (**Table 1**), the sections were incubated overnight at 4°C with each of the primary antibodies and the EnVision + system (DAKO, Glostrup, Denmark) was then applied. Negative controls were produced by substituting the primary antibody with nonimmunized serum.

Statistical analysis

Statistical significance was determined using the Chi-square. The level for significance was $P<0.05$.

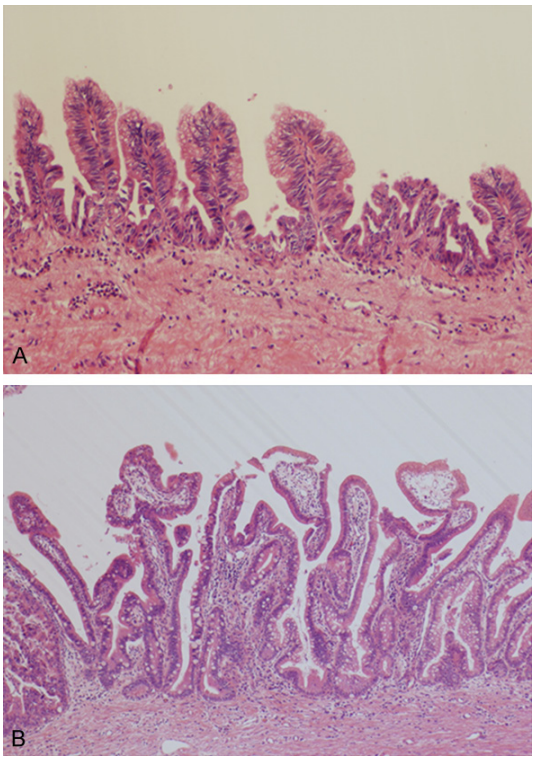

Figure 2. Intraepithelial spreading neoplasm of micropapillary type. A: Lower micropapillary type of gastric type. H&E, ×250 (original magnification). B: Higher micropapillary lesions of intestinal type. H&E, ×100 (original magnification).

Results

Main clinicopathological features

Fifty-two cases of IPNB were composed of 35 males and 17 females with age range from 37 to 84 years (mean 70.5 years). Main location of IPNB was intrahepatic large bile ducts (13 cases), perihilar bile ducts (19 cases), distal bile duct (19 cases) and anastomotic region between bile duct and jejunum (one case). Twenty-three cases of NS-CCA with IESN affecting the perihilar (17 cases) and distal bile ducts (6 cases) showed comparable sex (15 males and 8 females) and age (40-84 years, mean; 71 years) distribution of IPNB.

IESN and IPNB

Histologies and phenotypes

Histologies: In all cases of IPNB, IESN was found variably on the biliary mucosa along the proximal and distal bile ducts from IPNB lesions (**Figures 1-3**). The length of IESN from the edge

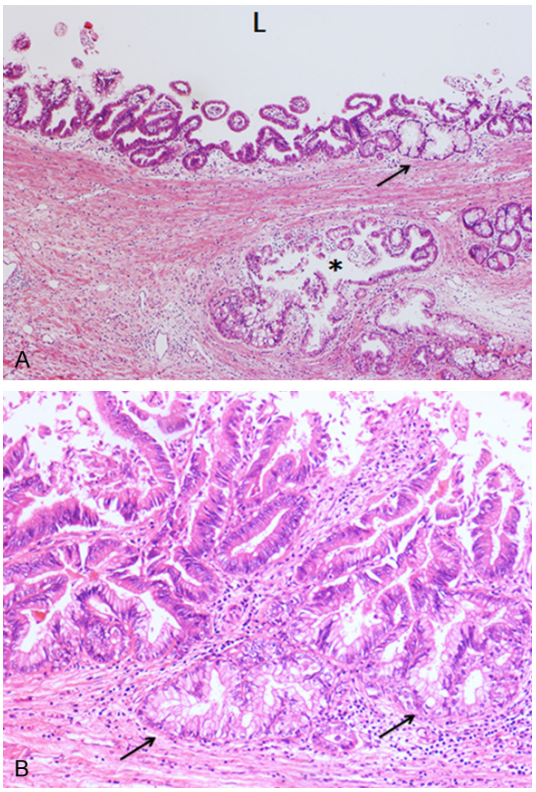

Figure 3. Intraepithelial spreading neoplasm with glandular formation. A: Lower micropapillary type of gastric type is found on the biliary mucosa of the bile duct. Similar intraepithelial neoplastic lesions are also found in the peribiliary glands (*) implicating involvement of peribiliary glands. Arrow, micropapillary lesion associated with glandular element (two stories-like). L, bile duct lumen. H&E, ×150 (original magnification). B: Micropapillary type associated with neoplastic glandular structures (arrows) (two stories-like). This combination recapitulates gastric foveola with pyloric glands. H&E, ×200 (original magnification).

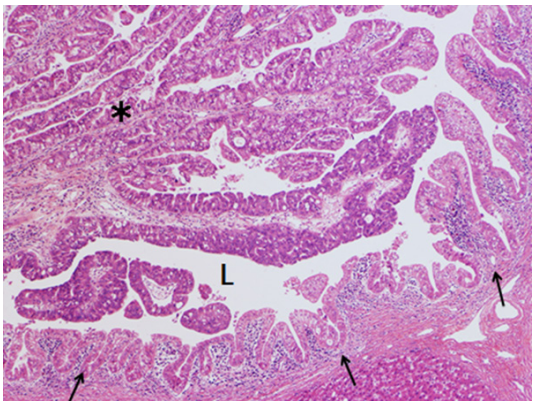

Figure 4. Intraductal papillary neoplasm of bile duct (IPNB) and adjacent intraepithelial spreading neoplasm (IESN). IPNB (*) and IESN (arrows) are continuous to each other, and show oncocytic type with some features of gastric type. L, bile duct lumen. H&E, ×150 (original magnification).

Table 2. Subtypes of intraepithelial spreading neoplasm (IESN) in intraductal papillary neoplasm of bile duct (IPNB) and nodular scerlosing cholangiocarcinoma (NS-CCA)

	IPNB (n-52*)	NS-CCA (n-23)
Subclassification of LSIN		
Flat type	52	23
Micropapillary type		
Low	52	12
High	43	0**

*, Number of cases; **, While three cases showed scattered "higher micropapillary type", they were not regarded as true higher micropapillary type (see the text). The incidence of LSIN is higher in IPNB (100%, 52 of 52 cases) than in NS-CCA (43.4%, 23 of 53 cases).

of grossly identifiable papillary tumor was more than 10 mm, and in extensive cases, IESN spread into the intrahepatic small bile ducts and also into the ampulla of Vater. IESN frequently involved the nearest peribiliary glands variably in all cases (**Figure 3A**, **3B**). Neoplastic epithelia of IESN were continuous of neoplastic epithelia lining IPNB (**Figure 4**).

Histological classification of IESN: IESNs were histologically classifiable into three lesions; flat, and lower and higher micropapillary types. Incidence of these types in IPNB and NS-CCA (see below) are shown in **Table 2**. In IPNB, these three types were variably distributed in individual cases, and they were continuous to each other.

Flat type: This type was characterized by intraepithelial spread of neoplastic epithelial cells showing flat or waving pattern (**Figure 1A**). Pseudopapillary pattern without fibrovascular core or stroma was also included in this type (**Figure 1B**). This type was identifiable in IESN of all cases of IPNB.

Micropapillary type (lower and higher): IESN showing micropapillary configurations with small amount of fibrovascualr stroma was frequently found, though they were usually less than 3 mm in their height from the basal lamina of bile duct. They were subdivided into lower (lower than 300 μm) (**Figure 2A**) and higher micropapillary (higher than 300 μm) types (**Figure 2B**). Lower type was found in all cases

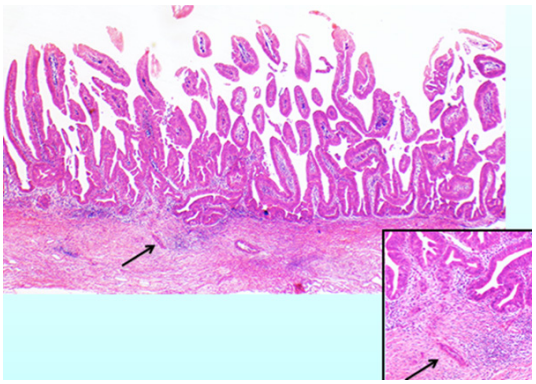

Figure 5. Intraepithelial spreading neoplasm of higher micropapillary type with invasion. Arrows show invasion. Inset is a higher magnification of invasion. H&E, ×100 (original magnification).

of IPNB, and higher type was found in 43 of 52 cases (82.7%).

Interestingly, 21 of 52 cases of IPNB showed considerable spread of IESN of higher micropapillary type on the biliary mucosa, and some micropapillary lesions were higher than 3 mm but less than 5 mm beside IPNB lesions in 3 cases.

Interestingly, miropapillary type, particularly higher one, frequently showed "two storied-structures" (**Figure 3**). Upper-story of neoplastic cells was papillary pattern, while the lower story of neoplastic cells showed as glandular or tubular structures. They resembled non-neoplastic structures of gastrointestinal tract such as the gastric foveola and small intestinal villi (mature or immature): others resembled villous or papillary adenoma or well-differentiated adenocarcinoma of gastrointestinal tract.

Subtypes: IPNB and IESN were classifiable into either of four subtypes, based on the predominant subtype, though more than two subtypes were encountered frequently in a single case or lesion. As for IPNB, the intestinal type was found in 29 cases followed by the gastric type (10 cases), PB type (7 cases) and oncocytic type (5 cases). The remaining one case was unclassifiable due to extensive mixture of four phenotypes in a single papillary lesion. The phenotypes of IESN were similar to those of IPNB in individual cases.

Grades and invasion of IESN of IPNB

Grades: As for the grade of IPNB, 5 cases were of low/intermediate grade, 15 cases of high grade without invasion, and the remaining 32 cases of high grade with invasion. The grade(s) of IESN were found to be similar to those of IPNB in individual cases. The grades of IESN were variable from area to area in the same case and even in the same lesion; some areas seemed to correspond to low/intermediate grade, and other were corresponding to high grade.

Invasive lesions at IESN and at IPNB: Invasion of carcinoma cells into the duct wall and beyond was found in one to several foci at IESN(s) in 22 cases of 52 cases of IPNB (**Figure 5**). Such invasion was frequently found in the area showing the micropapillary (lower and higher) types (21 cases) but rare in the flat type (2 cases). Invasion was found at IESN distant from IPNB, while invasion was also found at IESN near but not continuous with IPNB. Interestingly, invasion was found at high grade IESN, but not at low/intermediate IESN. As for the depth of invasion, 18 cases were of T1, and 4 cases of T2.

While invasive carcinoma of all cases showed tubular or cord-like carcinoma, two of them showed additional mucinous carcinoma. Irrespective of subtype(s) of IPNB and IESN, invasive parts usually shared expression of MUC1 and CK7 in addition to the focal or infrequent expression of other markers such as CK20 and MUC5AC.

As for the correlation between the invasion into the ductal wall and periductal tissue at the sites of IESN and/or at the bottom of IPNB, the invasion was found in 32 of 52 cases of IPNB. The occurrence of invasive lesions at IESN was found in 19 cases (59.3%) of 32 cases with invasion at the bottom of IPNB, while such invasion was found only in 3 cases (15%) of 20 cases without invasion at the bottom of IPNB ($P<0.05$) (**Table 3**), suggesting that the invasion was likely to occur at IESN and also IPNB in the same cases, and that intraepithelial neoplasm of IESN and IPNB were tended to have similar invasive potential.

NS-CCA

IESN of 23 cases of NS-CCA showed flat type, and 12 of these cases showed additionally lower, micropapillary type (**Table 3**). Such neoplastic micropapillae on the luminal surface were rather sparse and focal in LSIN of NS-CCA in comparison with those of IPNB. While 3 of

Table 3. Correlation between invasion at intraepithelial spreading neoplasm (IESN) and at intraductal papillary neoplasm of bile duct (IPNB)

		Invasion at main tumor of IPNB	
		No	Invasion
Invasion at IENB	No	•••••••••••• •••••••	•••••••••••• ••
	Invasion	•••	•••••••••••• ••••••••

these 12 cases showed also scattered "higher micropapillary type", they were thought to intraepithelial neoplastic replacement of non-neoplastic papillae of biliary mucosa and were different from higher micropapillary pattern as seen in LSIN of IPNB. In addition, the degree of cellular and nuclear atypia was high and rather homogeneous in LSIN of NS-CCA in comparison with those of IPNB. As for the subtypes of the main tumor of NS-CCA, all were of PB type, and IESN of NC-CCA was regarded as PB type, though there were also focal or infrequent expression of gastric or intestinal markers in NC-CCA and also IESN. However, there were no cases of oncocytic type in NS-CCA or IESN.

Discussion

The main findings of this study were summarized as follows: i) IPNB was constantly associated with LSIN, and IESN and IPNB showed the same subtype(s) and similar biological and pathological behaviors in individual cases. ii) Invasive lesion(s) were found at IESN in 22 of and also at IPNB in 32 of 52 cases, and invasion was tended to be found at both lesions in the same cases. iii) Four phenotypes were found in IPNB and LSIN in IPNB, while only PB type was found in NS-CCA and IESN in NS-CCA. iv) IESN of IPNB was classifiable histologically into flat, and lower and higher micropapillary types, while IESN in NS-CCA was classifiable only into flat and low micropapillary types. These data suggest that IPNB and IESN of IPNB show similar biological and pathological features in individual cases.

There have been several reports that intraductal papillary carcinoma of bile duct (IPNB with an associated invasive carcinoma) was frequently associated with IESN in comparison with other gross types of CCA [14, 15]. It was found in this study that IESN was found in all cases of IPNB and the extent of LSIN was variable. Three types of IESN (flat, and low and higher micropapillary types) were usually found in variable combinations in individual cases of IPNB; the flat and lower micropapillary types were found in all cases, and higher micropapillary type was also frequent, and they were continuous to each other. Among these three structures, micropapillary type, particularly higher one, resembles the gastrointestinal mucosa, and also papillary and villous adenoma and carcinoma of the gastrointestinal tract. It is possible that these unique histological structures of higher micropapillary type of LSIN reflect the intestinal and gastric cell lineages or phenotypes.

There have been many reports that IPNBs occur multifocally, synchronously or metachronously, suggesting that IESN of IPNB may grow and eventually become grossly visible papillae (IPNB) [21-23]. Interestingly, higher micropapillary types were rather extensive in 21 cases of IPNB, and these 21 cases could belong to the previously used entity of "biliary papillomatosis" [21-23].

IPNB and IESN showed four subtypes (intestinal, gastric, PB and oncocytic types), and the subtypes of IESN were similar to those of IPNB in individual cases.

The grade of cellular and nuclear atypia of IESN was also variable or heterogeneous from area to area in individual cases. These heterogeneous grades were constantly found in IESN lesions irrespective of the above-mentioned three histological features and subtypes. However, the grades of intraepithelial neoplasia of IESN were in the ends evaluated to be similar to that of IPNB in individual cases, suggesting that the heterogeneous grades were also shared by IESN and IPNB in individual cases. Such heterogeneity of grades of single case or even in single lesion were already described in IPNB lesions [13], and also in papillary carcinoma of gallbladder, extrahepatic bile ducts and ampulla of Vater which is now included in IPN of biliary tract [24-26].

IPNB is well known to be eventually followed by invasive CCA (IPNB with an associated invasive carcinoma) [1]. It was found in this study that

invasion of carcinoma into the ductal wall was found at the sites of IESN in addition to the site of IPNB. Among three subtypes of IESN, a majority of invasion tended to develop at the micropapillary type of IESN, while such invasion was rare in the flat type. There were evident fibrovascular connective tissues in the micropapillary type, particularly higher ones associated with neoplastic glandular formation, and this environment may be favorable for invasion of carcinoma cells into the ductal wall [27, 28]. Interestingly, the invasion at the sites of IESN was frequently associated with the invasion of carcinoma at the bottom of IPNB lesions in the same cases ($P<0.05$), suggesting that the invasion of carcinoma occur multifocally at the bottom of IPNB and also at the sites of IESN, and that IESN has similar invasive potential as IPNB. IPMNs are also known to show unifocal and multifocal invasion at laterally spreading lesion and/or main papillary lesion [10].

By considering all of these findings that IPNB and IESN share similar biological characters and pathological behaviors in individual cases and that the lining epithelia of IPNB and IESN were continuous to each other, it seems plausible that IPNB and IESN compose of a unique intraepithelial neoplasm of bile duct followed by invasion. However, it remains speculative whether IESN precedes IPNB or IPNB precedes IESN or both occur simultaneously.

IESN is known in perihilar and distal NS-CCA. The frequency of IESN in NC-CCAs is reported as low in comparison with other gross types of CCA by Sakamoto [14] and also Nakanishi [15]. As for the structures of IESN of NS-CCA, it was found that only flat type and to a lesser frequency lower micropapillary type was found, suggesting that higher micropapillary was rather unique to IESN of IPNB. As for the subtypes, main tumor of NS-CCA was of PB type, and IESN of 23 cases of NC-CCA were also of PB type, though they also presented focal or weak gastric or intestinal markers such as MUC2 and MUC5AC. In this context, four phenotypes and higher micropapillary type of IESN of PNB seem to highlight the characteristic features inherent in IPNB itself.

In conclusion, IESN was found in all cases of IPNB, and higher micropapillary type of LSIN and four phenotypes were rather unique to IPNB. The types of phenotype, heterogeneous grades of atypia, and potential of invasion of IESN were similar in those of IPNB itself in individual cases, suggesting that IESN and IPNB compose of unique intraepithelial neoplasm of the same character of bile duct. Practically, IESN of IPNB has an imminent invasive potential as in IPNB, and clinician should be aware of IESN as a potential lesion of invasion in cases of IPNB during operation or endoscopic manipulation.

Disclosure of conflict of interest

None.

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