



## Outcomes of surgery for 2010 WHO classification-based intraductal papillary neoplasm of the bile duct: Case–control study of a single Japanese institution's experience with special attention to mucin expression patterns



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### ABSTRACT

**Introduction:** The World Health Organization (WHO) proposed an integrated classification for intraductal papillary neoplasm of the bile duct (IPNB) in 2010. However, IPNB reportedly shows considerable geographic variation. This Japanese single-institution study examined outcomes of surgery for IPNB and the prognostic impact of immunohistochemical mucin expression patterns.

**Materials and methods:** Patients with IPNB were identified from 413 patients who underwent curative-intent surgery for biliary tract (excluding gallbladder) neoplasms from 1992 to 2016 by retrospective macro- and microscopic reevaluation of resected specimens. Their clinicopathological variables were analyzed.

**Results:** Twenty-two (5%) 2010 WHO classification-based patients with IPNB were identified. The other 391 patients had common-type cholangiocarcinoma. The histopathological grade was low/intermediate in 2 patients (9%), high in 8 (36%), and invasive carcinoma (ICa) in 12 (55%). The 10-year overall survival rate was 100% in 10 patients with low–high grade IPNB and 69% in 12 patients with ICa. These rates were significantly ( $p=0.018$ ) or marginally ( $p=0.089$ ) better than that (38%) of 391 other-cholangiocarcinoma patients. In the 12 patients with ICa, R0 or R1 resection, MUC5AC, and MUC6 expression significantly affected survival. Notably, all seven patients with ICa exhibiting MUC5AC expression survived throughout the study period, while four of five patients with ICa who did not exhibit MUC5AC expression died of recurrence (with vs. without MUC5AC: 10-year overall survival, 100% vs. 60%, respectively;  $p=0.018$ ).

**Conclusion:** Our 24-year, single institution's experience suggests that Japanese patients with IPNB favorably respond to surgery, even with ICa. MUC5AC and MUC6 expression may be predictive of favorable outcomes.

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### Introduction

Intraductal papillary neoplasm of the bile duct (IPNB) might have historically included various different disease entities because a uniform classification did not exist until the World Health

Organization (WHO) proposed an integrated classification, recognizing IPNB as a distinct entity, in 2010 [1–3]. IPNB has been considered a biliary counterpart to intraductal papillary mucinous neoplasm of the pancreas (P-IPMN) [4]. However unlike P-IPMN, for which a therapeutic guideline has already been established [5], the clinicopathological features of IPNB have remained unclear due to its rareness. A recent meta-analysis that attempted to characterize the clinical features of IPNB recommended early surgery for radiologically suspected IPNB [6]. This recommendation was based

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on the poor sensitivity of endoscopic biopsy for the diagnosis of invasive disease in patients with IPNB despite the fact that at least 43% of specimens from 476 patients undergoing curative-intent surgery contained invasive disease [6]. However, most cases included in this meta-analysis were diagnosed before the establishment of the 2010 WHO classification. Although several studies regarding surgical outcomes of 2010 WHO classification-based IPNB were performed in the USA, South Korea, and Thailand [7–11], the clinicopathological characteristics of IPNB should still be considered unclear because the pathobiological behavior of IPNB reportedly demonstrates considerable geographic variation [6–11].

The present study examined a single Japanese institution's experience from 1992 to 2016 to determine the outcomes of surgery for IPNB diagnosed by the 2010 WHO classification. We also examined the prognostic impact of mucin expression patterns, of which the prognostic significance has been reported in mucin-producing tumors of other organs including P-IPMN [4,12–14], on IPNB.

## Materials and methods

### Patients

The present study was approved by the Yokohama City University ethics committee. Patients with IPNB were identified by retrospective macro- and microscopic reevaluation of resected specimens among 413 consecutive patients who underwent curative-intent surgery for biliary tract tumors (excluding those of the gallbladder) from April 1992 to March 2016 at Yokohama City University Hospital. The following clinicopathological variables of patients with IPNB were retrospectively assessed: age at surgery, gender, location of the tumor, macroscopic mucin secretion, pre-operative pathological diagnosis, operative procedures, pathological findings of resected specimens, including mucin expression patterns, and the clinical course. Survival outcomes were compared between patients with IPNB and those with common-type cholangiocarcinoma. Furthermore, the prognostic impact of the above-mentioned clinicopathological variables on outcomes of surgery for IPNB was assessed. IPNB was diagnosed based on the 2010 WHO classification: macroscopic papillary growth in an intrahepatic or extrahepatic bile duct, regardless of mucin production, and the microscopic presence of intraductal papillary growth with delicate fibrovascular stalks [3]. Microscopic epithelial subtypes were classified into the following four types: gastric, intestinal, pancreatobiliary, and oncocytic types. The subtype of each case was adopted as the predominant subtype. Two surgeons (F.H. and R.M.) and one pathologist (S.Y.) reinvestigated the resected specimens to reach a consensus regarding the diagnosis of IPNB. Several previous studies revealed that a positive margin is significantly disadvantageous to the outcomes of surgery for IPNB [6–11], although the margin status was not clearly defined in these reports. Because the present study cohort included patients with IPNB with a histological grade ranging from low-grade to invasive carcinoma, the margin status was defined as follows. When the most atypical histological grade in the resected specimen was low-to high-grade (i.e., carcinoma in situ [CIS]), the margin status was judged negative if no atypical cells or cells showing less atypia than the most atypical histological grade in the resected specimen were observed at the resection margin. In cases of associated invasive carcinoma, a positive margin was defined as a margin showing positivity for CIS or invasive carcinoma. Furthermore, the proportion (%) of the invasive carcinoma component in an entire tumor was assessed in patients with associated invasive carcinoma because Jarnagin et al. [15] reported that an invasive carcinoma component of  $\geq 10\%$  was significantly disadvantageous to postoperative survival in patients

with papillary phenotype cholangiocarcinoma, which was considered to be IPNB associated with invasive carcinoma. American Joint Committee on Cancer (AJCC) staging [16] was applied to patients who showed associated invasive carcinoma.

### Immunohistochemistry

We assessed the immunohistochemical expression patterns of MUC1, MUC2, MUC5AC, and MUC6. Four-micrometer-thick sections fixed in 4% buffered formaldehyde and embedded in paraffin were deparaffinized in xylene and rehydrated in alcohol. Antigen retrieval was carried out via autoclave pretreatment (121 °C, 10 min) in citrate buffer (pH 6). The endogenous peroxidase activity was blocked by soaking the specimens in methanol containing 0.03% hydrogen peroxide for 30 min at room temperature. The specimens were then incubated sequentially with 10% rabbit serum albumin and the primary antibodies (37 °C, 60 min). The primary antibodies were MUC1 (clone Ma695, dilution 1:100; Novocastra, Newcastle, UK), MUC2 (clone Ccp58, dilution 1:100; Novocastra), MUC5AC (clone CHL2, dilution 1:100; Novocastra), and MUC6 (clone CHL5, dilution 1:100; Novocastra). Immunohistochemical reactions were visualized with a HistoFine kit (Nichirei Pharmaceutical, Tokyo, Japan) and DAB system (Dako, Carpinteria, CA, USA). A percentage of positive cells exceeding 5% was defined as positive, as in previous studies<sup>[13,14,17,18]</sup>.

### Statistical analyses

Fisher's exact test was used to compare nonparametric categorical variables. Mann–Whitney *U* test was used to compare nonparametric continuous variables. Follow-up of the patients was continued until December 31, 2016 or until the patient's death. Primary outcomes were disease-free survival (DFS) and overall survival (OS). DFS was defined as the duration from the date of surgery to the day of diagnosing relapsed disease or the latest follow-up. OS was defined as the duration from the date of surgery to the day of the patient's death or the latest follow-up. The survival rate was calculated by the Kaplan–Meier method, and the Breslow–Gehan–Wilcoxon test was used for comparison among groups. A *p*-value of  $<0.05$  was considered significant. All analyses were performed with SPSS software ver. 21 (IBM Corp., Armonk, NY, USA).

## Results

### Patients' characteristics

Among the 413 patients who underwent curative-intent surgery for biliary tract tumors, 22 (5%) met the 2010 WHO criteria for IPNB. The remaining 391 patients had common-type cholangiocarcinoma. In the 22 patients with IPNB, the postoperative observational period ranged from 15 to 192 months with a median of 49 months. The main location of the tumor was intrahepatic in 12 patients (55%) and extrahepatic in 10 (46%). Macroscopic mucus secretion was observed in 12 patients (55%). Surgical procedures included hepatectomy in 19 patients (86%) (hemihepatectomy, 16; sectionectomy, 3), pancreaticoduodenectomy with or without hilar bile duct resection in 2 (9%), and hilar bile duct resection alone in 1 (5%). Eleven (58%) of the 19 patients who underwent simultaneous hepatectomy also underwent extrahepatic bile duct resection and reconstruction (Table 1).

### Pathological features

The histopathological grade was low or intermediate (benign) in

**Table 1**  
Patients' demographics and clinical characteristics.

Variable	
Age (years), median (range)	69.5 (53–86)
Gender, n	
Male	13
Female	9
Follow-up period (months), median (range)	49 (15–192)
Tumor location, n	
Intrahepatic bile duct	12
Perihilar/Distal bile duct	10
Macroscopic mucus secretion, n	
Present	12
Absent	10
Operation, n	
Hemihepatectomy	16
Sectionectomy	3
Hilar bile duct resection	1
Hilar bile duct resection + Pancreaticoduodenectomy	1
Pancreaticoduodenectomy	1
Bile duct resection in hepatectomy cases (n = 19), n	
Present	11
Absent	8

2 patients (9%) and high (CIS) in 8 (36%), and associated invasive carcinoma was observed in 12 (55%). Notably, a preoperative histological diagnosis of adenocarcinoma was achieved in only 3 (30%) of the 10 patients with invasive carcinoma who underwent preoperative cytology or biopsy under cholangiography or via cholangio-endoscopy. Lymph node metastasis, microvascular invasion, or perineural invasion was not detected in any patients. Curative resection (i.e., R0 resection) was achieved in 19 patients (86%). The remaining three, all of whom exhibited invasive carcinoma, achieved R1 resection: two patients exhibited microscopic evidence of invasive carcinoma, and the remaining patient exhibited CIS at the resection margin.

The subtype was pancreatobiliary in 10 (46%) patients, intestinal in 7 (32%), oncocytic in 4 (18%), and gastric in only 1 (5%) (Fig. 1a–d). No significant association was found between the histological subtype and histological grade or the presence of invasive carcinoma (Table 2).

### Mucin expression

MUC1 was expressed in the cell membrane, whereas MUC2, MUC5AC, and MUC6 were expressed in the cytoplasm (Fig. 1e–h). MUC1-, MUC2-, MUC5AC-, and MUC6-positive tumors were present in 12 (55%), 9 (41%), 14 (64%), and 16 (73%) patients, respectively.

### Postoperative outcomes

Five patients (23%) developed recurrence. The time to first recurrence ranged from 2 to 151 months with a median of 25 months. Four of the five patients who developed recurrence had associated invasive carcinoma. The remaining patient underwent left hepatectomy with extrahepatic bile duct resection for intermediate-grade IPNB (R0 resection) and thereafter developed recurrent intermediate-grade IPNB in the intrapancreatic residual bile duct 77 months after hepatectomy (Table 2). The patient underwent pancreaticoduodenectomy for the recurrence and remained clinically well without re-recurrence for 15 years after the initial surgery. All four patients who had associated invasive carcinoma and developed recurrence died during the study period.

The 5- and 10-year overall survival (OS) rates of all patients were 90% and 80%, respectively. Notably, none of the 10 patients with

low–high grade IPNB died during the study period, while 4 of the 12 patients with IPNB with invasive carcinoma died of recurrence. However, postoperative survival did not differ between IPNB patients with and without invasive carcinoma ( $p = 0.155$ ). The 5- and 10-year OS rates were significantly higher in the 10 patients with low–high grade IPNB than in the patients with other cholangiocarcinomas (5-year, 100% vs. 45%; 10-year, 100% vs. 38%;  $p = 0.009$ ) (Fig. 2a). Furthermore, these rates tended to be better in the 12 patients with IPNB with invasive carcinoma than in the patients with other cholangiocarcinomas, although the difference did not reach statistical significance (5-year, 83% vs. 45%; 10-year, 69% vs. 38%;  $p = 0.089$ ) (Fig. 2a).

### Prognostic factors for postoperative survival

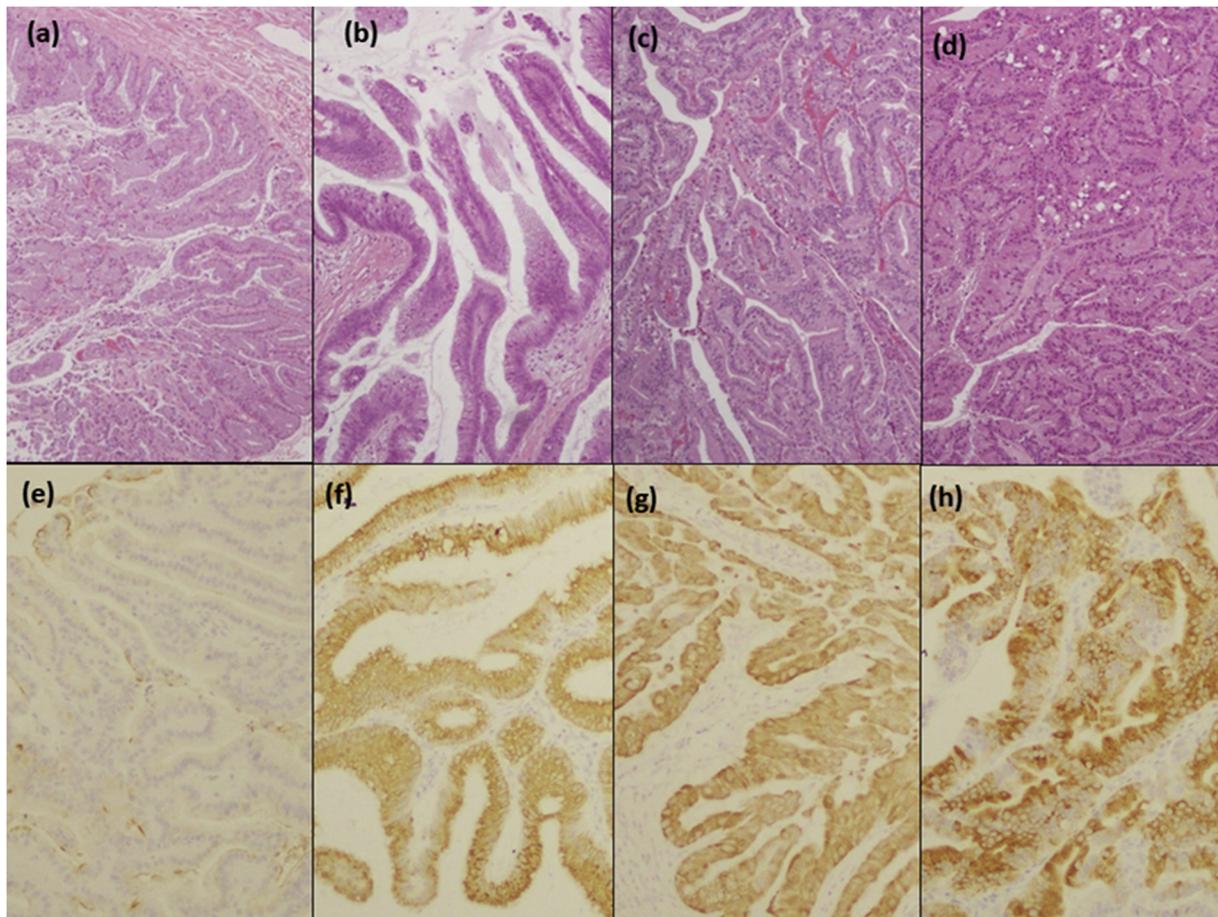
Because none of the 10 patients with low–high grade IPNB died during the study period, prognostic factors were sought only for the 12 patients with associated invasive carcinoma. The margin status significantly affected survival (R0 vs. R1 resection: 3-year OS rate, 100% vs. 33%;  $p = 0.010$ ) (Fig. 2b). However, comparison of clinicopathological variables, including mucin expression patterns, between the R0 ( $n = 9$ ) and R1 ( $n = 3$ ) resection groups did not show any significant factors predictive of R1 resection. Unlike the above-mentioned study by Jarnagin et al. [15], the proportion of the invasive carcinoma component did not significantly affect survival. Three of the 12 patients with invasive carcinoma showed <10% of the invasive carcinoma component, whereas the remaining nine patients showed  $\geq 50\%$  of the invasive carcinoma component. Five- and 10-year OS rates were similar between the former and latter groups (<10% vs.  $\geq 50\%$ ; 5-year OS rate, 100% vs. 78%; 10-year OS rate, 100% vs. 60%;  $p = 0.3865$ ).

Expression of MUC1 or MUC2 did not significantly impact the survival outcomes (data not shown). Disease-free survival (DFS) and OS were significantly better in patients with than without MUC6 expression, although the longest observational period of patients without MUC6 expression was very short at <3 years (DFS,  $p = 0.027$ ; OS,  $p = 0.010$ ) (Fig. 3c and d). Among MUC1, MUC2, MUC5AC, and MUC6, MUC5AC had the most prominent and significant impact on survival outcomes. The 5-year DFS and OS rates were significantly higher in patients with than without MUC5AC expression (10-year DFS rate, 100% vs. 40%,  $p = 0.014$ ; 10-year OS rate, 100% vs. 60%,  $p = 0.028$ ) (Fig. 3c and d).

Comparison of the clinicopathological variables between patients with and without MUC5AC expression showed no differences in these variables with the exception of mucus secretion (71% vs. 25%,  $p = 0.048$ ) (Table 3). Although the margin status was found to have a significant impact on outcomes, the R0 resection rate as well as the AJCC staging did not differ between patients with and without MUC5AC expression. However, all seven patients with invasive carcinoma who exhibited MUC5AC expression survived throughout the study period, while four of the five patients with invasive carcinoma who did not exhibit MUC5AC expression died during the study period (Fig. 3a and b).

### Discussion

This 24-year, Japanese, single institution's experience showed that the outcomes of surgery for patients with 2010 WHO classification-based IPNB were favorable in terms of better survival outcomes compared with those of patients with other common-type cholangiocarcinomas. The present study also showed that lymph node metastasis, microvascular invasion, and perineural invasion were absent even in patients with invasive carcinoma, although we identified associated invasive carcinoma in most of the resected specimens. However, preoperative acquisition of the



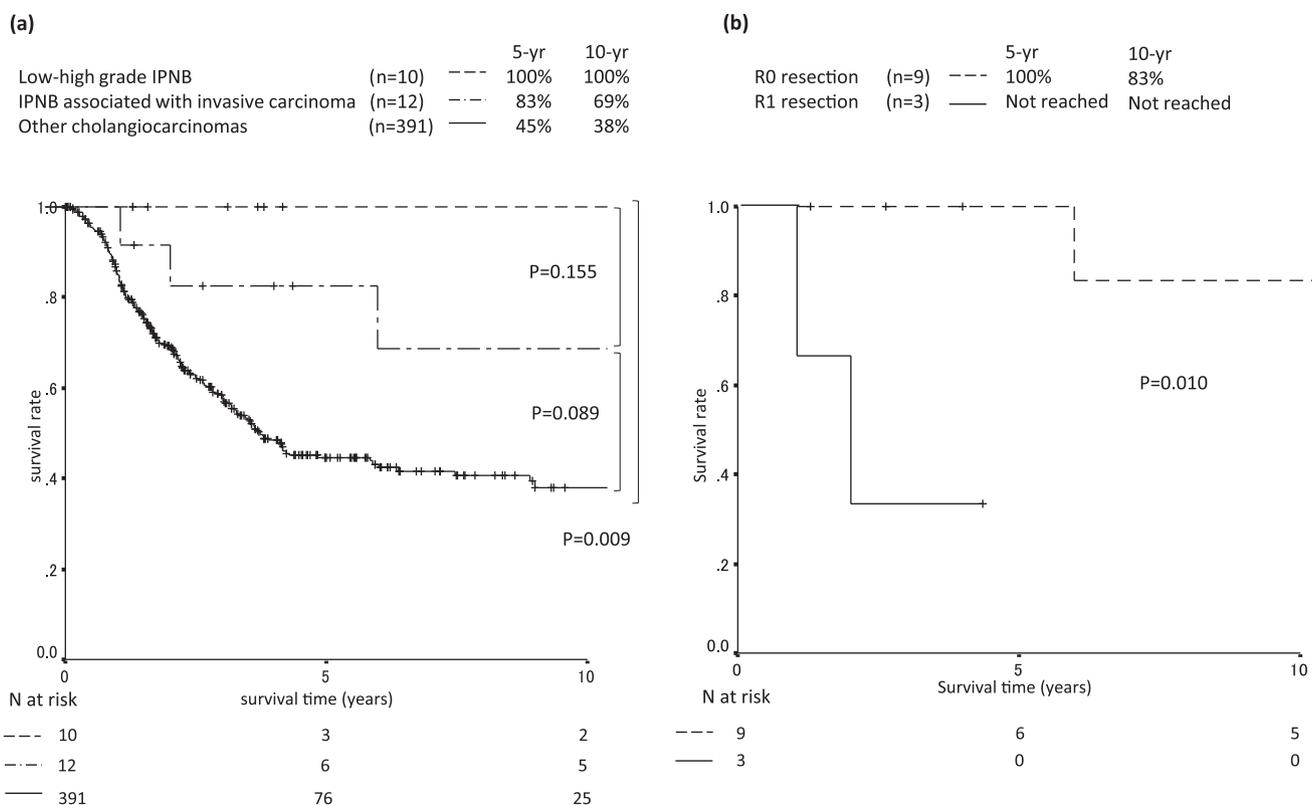
**Fig. 1.** Representative microscopic findings of each subtype of intraductal papillary neoplasms of the bile duct and immunohistochemical staining for MUC1, MUC2, MUC5AC, and MUC6.

Microscopic findings of (a) gastric type, (b) intestinal type, (c) pancreatobiliary type, and (d) oncocytic type (H&E stain,  $\times 100$ ). The most common subtype was pancreatobiliary ( $n = 10$ ), followed by intestinal ( $n = 7$ ) and oncocytic ( $n = 4$ ). The gastric subtype was found in only one patient. Immunopathological stains of (e) MUC1, (f) MUC2, (g) MUC5AC, and (h) MUC6 are shown ( $\times 100$ ). MUC1-, MUC2-, MUC5AC-, and MUC6-positive tumors were present in 12 (55%), 9 (41%), 14 (64%), and 16 (73%) patients, respectively.

**Table 2**  
Pathological features and Postoperative Outcome.

Variable	
Preoperative pathological diagnosis in invasive carcinoma cases ( $n = 12$ ), n	
None or unknown	2
Benign	5
Suspicious of malignancy	2
Adenocarcinoma	3
Postoperative histopathological grade, n	
Low/Intermediate (Benign)	2
High (Carcinoma in situ)	8
Invasive	12
Invasive component <10%	3
>10%	9
Tumor size (cm), median (range)	2.5 (1.3–7.0)
Lymph node involvement, n	0
Vascular invasion, n	0
Perineural invasion, n	0
Curability, n	
Curative (R0) resection	19
Ductal margin positive	2
Radial margin positive	1
Recurrence, n	5
Time to recurrence (mo), median (range)	47 (2–151)
Site of Recurrence, overlapped, n	
Local	4
Lymph node	1
Lung	2
Pleural and peritoneal dissemination	1

diagnosis of adenocarcinoma was achieved in only 3 of the 12 patients with associated invasive carcinoma. These results support the finding of the meta-analysis proposing early surgery for radiologically suspected IPNB [6]. Furthermore, the survival outcomes in the present study seemed remarkably better than those of previous reports [6–11], which may indicate geographic variation of IPNB. In addition, the resection margin and MUC5AC and MUC6 expression had a significant impact on survival outcomes in the present study. Several studies based on the 2010 WHO classification identified a margin status as an important prognostic factor for surgical outcomes [7–11]. MUC1 expression is reportedly significantly associated with the presence of associated invasive carcinoma and/or worse survival outcomes [6–8]. The present study was the first to show a significant correlation between survival outcomes and MUC5AC or MUC6 expression in patients with IPNB. Many studies have focused on the expression of various mucins, including MUC5AC and MUC6, in several digestive neoplasms [13,14,17–35]. The prognostic significance of these mucins varies among these studies. Furthermore, the prognostic significance of MUC6 expression has not been given special attention. However, discrepant results have been reported between the clinical impact of MUC5AC expression on surgical outcomes of cholangiocarcinoma and that of pancreatic neoplasms including P-IPMN; i.e., MUC5AC expression was reportedly associated with a high AJCC T category [27], lymph node metastasis [17], and worse

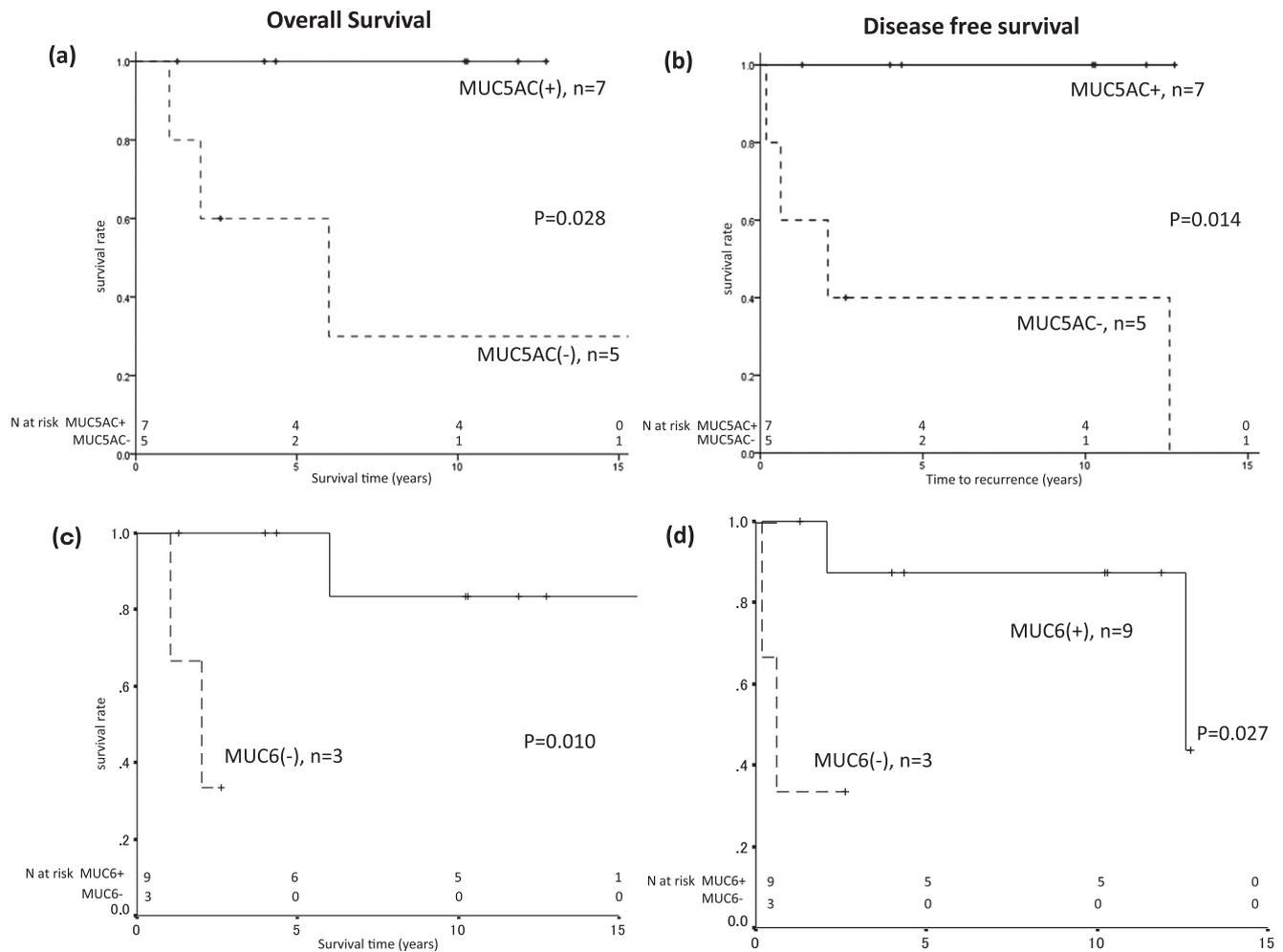


**Fig. 2.** (a) Comparison of postoperative overall survival among patients with low-to high-grade intraductal papillary neoplasm of the bile duct (IPNB), patients with IPNB with associated invasive carcinoma, and patients with common-type cholangiocarcinoma. (b) Impact of the margin status on postoperative survival of the 12 patients with associated invasive carcinoma.

The 5- and 10-year overall survival rates were significantly higher in patients with low–high grade IPNB than in patients with other cholangiocarcinomas (5-year, 100% vs. 45%; 10-year, 100% vs. 38%;  $p = 0.009$ ). Furthermore, these rates tended to be higher in patients with IPNB with invasive carcinoma than in patients with other cholangiocarcinomas, although the difference did not reach statistical significance (5-year, 83% vs. 45%; 10-year, 69% vs. 38%;  $p = 0.089$ ). However, postoperative survival did not differ between patients with IPNB with and without invasive carcinoma ( $p = 0.155$ ) (a). The positive margin status had a significantly worse impact on postoperative survival after surgery for the 12 patients with associated invasive carcinoma (b).

postoperative survival [17] in cholangiocarcinoma but was associated with decreased lymph node metastasis [29], a decreased incidence of vascular invasion [29,32], and better postoperative survival [14,29,32] in pancreatic neoplasms, including P-IPMN. MUC5AC reportedly weakens cell adhesion and enhances cell migration, leading to a robust metastatic capacity in cholangiocarcinoma [17,27,28]. In contrast, it is reportedly correlated with an expansive growth pattern and is considered unlikely to invade adjacent structures of pancreatic ductal adenocarcinoma as well as P-IPMN [29,32]. Among the 12 patients with invasive carcinoma in the present study, 4 of the 5 patients without MUC5AC expression died of recurrence, although all remaining 7 patients who exhibited MUC5AC expression survived throughout the study period. These findings regarding MUC5AC expression in the present study may indicate a biological similarity between IPNB and P-IPMN. Given the widespread use of high-quality cross-sectional imaging, however, P-IPMN is often encountered in daily clinical practice. Abundant clinical experience has therefore been accumulated, leading to the consensus of regarding P-IPMN as a distinct entity as well as the establishment of a therapeutic guideline [5]. In the guideline, the histological features or potential malignancy of P-IPMN can be speculated to a certain degree of accuracy according to the diagnostic imaging findings. Although much room remains for increased accuracy, the optimal therapeutic strategy for each case of P-IPMN can be determined based on the guideline. Unlike P-IPMN, the correlation between the diagnostic imaging findings and histological grade of IPNB has been infrequently examined because

of the rarity of IPNB. A recently published statement for consensus building of IPNB referred to the imaging findings of IPNB [36]. In this statement by Nakanuma et al. [36], IPNB can be classified into the following 2 subtypes: P-IPMN-like and P-IPMN-unlike subtypes. The P-IPMN-like subtype literally resembles P-IPMN in histopathology. This subtype commonly develops in the intrahepatic bile duct and shows gross mucin secretion, thin fibrovascular stalks, and edematous stroma in most cases [36]. Therefore, the P-IPMN-like subtype typically appears to show remarkable cystic dilatation of the intrahepatic bile duct without an obvious intraductal solid component in cross-sectional imaging. The P-IPMN-unlike subtype typically indwells in the extrahepatic bile duct including the hilar duct. This subtype rarely shows gross mucin secretion. The P-IPMN-unlike subtype usually shows papillary growth with fibrovascular stalks that are typically >5 mm in height from the neighboring biliary mucosa [36]. In diagnostic imaging, typical cases of the P-IPMN-unlike subtype appear to be recognized as tumors showing well-defined intraductal papillary growth in the extrahepatic bile duct that are well enhanced in contrast-enhanced imaging. Furthermore, approximately 50% of cases of the P-IPMN-like subtype show an invasive carcinoma component, while more than 90% of cases of the P-IPMN-unlike subtype show associated invasive carcinoma [36]. In cases of P-IPMN, clinical findings that are highly suggestive of malignancy, such as high-risk stigmata or worrisome features, have been determined with abundant accumulation of case experience. These cases should undergo surgery [37]. If such clinical features strongly suggestive of associated invasive



**Fig. 3.** Comparison of postoperative overall and disease-free survival between patients with and without MUC5AC or MUC6 expression among all patients with associated invasive carcinoma.

(a, b) Among the 12 patients with associated invasive carcinoma, the 5-year DFS and OS rates were significantly higher in patients with than without MUC5AC expression (5-year DFS rate, 100% vs. 40%,  $p = 0.014$ ; 5-year OS rate, 100% vs. 60%,  $p = 0.028$ ). (c, d) Similarly, the 5-year DFS and OS rates were significantly higher in patients with than without MUC6 expression (DFS,  $p = 0.027$ ; OS,  $p = 0.010$ ), although the longest observational period for patients without MUC6 expression was short at <3 years.

**Table 3**  
Stratified according to expression of MUC5AC.

Variable	MUC5AC (+) (n = 14)	MUC5AC (-) (n = 8)	p-value
Tumor location, n			
Intrahepatic bile duct	9	3	0.221
Perihilar/Distal bile duct	5	5	
Macroscopic mucus secretion, n	10	2	0.048
Histopathological grade, n			
Low-high grade	7	3	0.675
Invasive carcinoma	7	5	
Histopathological subtype, n			
Pancreatobiliary	8	2	0.218
Intestinal	3	4	
Oncocytic	3	1	
Gastric	0	1	
Tumor size (cm), median (range)	2.9 (1.3–7.0)	2.5 (1.3–6.5)	0.736
Invasive component (n = 12), n			
<10%	3	0	0.159
>10%	4	5	
Curability (invasive carcinoma, n = 12), n			
R0 resection	6	3	0.523
R1 resection	1	2	
AJCC Stage (invasive carcinoma, n = 12), n			
I (Ia-Ib)	7	4	0.417
III	0	1	

carcinoma in cases of IPNB could be clarified with accumulation of cases, watchful observation with periodical diagnostic imaging checkups might be acceptable in selected cases of the P-IPMN-like subtype, such as in cases of P-IPMN [5]. At present, the associations between histopathological grades and findings of diagnostic imaging have not been well studied. Hence, multi-institutional accumulation of many more cases, including the findings of diagnostic imaging, is necessary to examine this issue, as well as arrive at a consensus regarding the diagnosis of IPNB.

This study has several limitations. First, several variabilities existed because of the lengthy study period of 24 years, although they were unavoidable due to the rarity of IPNB. These variabilities were caused by the remarkable refinements that have been undoubtedly achieved in surgical techniques/devices, diagnostic imaging, and patient management methods during the 24-year period. For example, the preciseness of diagnostic imaging absolutely differed between early cases and recent cases. Thus, the correlation between the diagnostic imaging findings and the histological grading was not examined. Second, because this was a single-institution study, the sample size was very small. Third, the expression of mucins was examined only with immunohistochemical staining. The long preservation time required for specimens of the early cases did not allow us to obtain specimens suitable for molecular analyses. In spite of these limitations, we believe that the present study is valid because it is the first report based on the 2010 WHO classification from Japan to focus on the impact of mucin expression patterns on surgical outcomes.

In conclusion, this 24-year, single institution's experience suggests that Japanese patients with IPNB based on the 2010 WHO classification favorably respond to surgery in terms of better survival outcomes compared with those of common-type cholangiocarcinomas. No lymph node metastasis, microvascular invasion, or perineural invasion was observed, even in patients with associated invasive carcinoma. Expression of MUC5AC and MUC6 may be predictive of favorable surgical outcomes.

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## Declarations of interest

None.

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