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Original contribution

Pancreatobiliary-type intraductal papillary mucinous neoplasm of the pancreas may have 2 subtypes with distinct clinicopathologic and genetic features☆

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Summary We recently experienced cases of pancreatobiliary-type intraductal papillary mucinous neoplasms (PB-IPMNs) with imaging features resembling pancreatic ductal adenocarcinomas (PDACs), and histologic appearance of purely pancreatobiliary morphology and highly distorted papillary growth, which led to the present study aiming to systematically investigate PB-IPMNs in comparison with PDACs. Surgical cases of PB-IPMNs (n = 31) and PDACs (n = 24) were examined. PB-IPMNs were classified into monotypic tumors (n = 12; 39%) consisting of entirely high-grade pancreatobiliary-type neoplastic cells and polytypic cases (n = 19; 61%) associated with components of low-grade dysplasia and/or other histologic types (eg, gastric, intestinal, or oncocytic types). Clinically, monotypic PB-IPMNs less commonly had dilatation of the ampullary orifice (0% versus 74%) and mucin hypersecretion (17% versus 89%) than did polytypic cases. In most cases of monotypic PB-IPMNs, cystic dilatation of the lesional ducts was less obvious on imaging; therefore, 33% were radiologically diagnosed as PDACs. Histologically, intraductal tumors in monotypic cases showed a highly complex papillary architecture with tubular/cribriform glands and irregular branching, and all these cases were associated with invasive malignancy. *GNAS* mutations were detected in polytypic PB-IPMNs (6/19; 32%), but there were no *GNAS* mutations in monotypic cases. The recurrence-free survival of patients with monotypic PB-IPMN or PDAC was similar and significantly worse than that of patients with polytypic PB-IPMN. In conclusion, some cases of monotypic PB-IPMNs lacked the classic characteristics of IPMNs and shared features with PDACs, raising the possibility that these cases may be better classified as a papillary variant of PDACs rather than IPMNs.

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1. Introduction

Intraductal papillary mucinous neoplasm (IPMN) of the pancreas is a distinct type of tumor characterized by duct dilatation, mucin overproduction, and potential progression to invasive ductal carcinoma [1,2]. It is classified into 3 histologic groups based on microscopic appearance, and the pathologic classification correlates well with clinical features [2,3]. The gastric type typically develops in branch ducts and has the lowest risk of malignant transformation [4]. Intestinal-type IPMNs present with dilatation of the main pancreatic duct with abundant mucin secretion [4]. This group sometimes exhibits features of mucinous carcinoma, when it progresses to invasive malignancy. Pancreatobiliary-type IPMNs (PB-IPMNs) usually involve the main pancreatic duct at least focally and have features of high-grade dysplasia. PB-IPMN is the least common and therefore the least well-characterized [3]. Intraductal oncocytic papillary neoplasms, also often referred to as oncocytic-type IPMNs, are currently considered to be a separate entity with unique microscopic features including complex and arborizing papillae with delicate cores, cuboidal to polygonal cells with abundant eosinophilic cytoplasm, and centrally located large nuclei with distinct nucleoli. Intraductal oncocytic papillary neoplasms are sometimes associated with other subtypes of IPMNs.

The genetic features of pancreatic IPMNs have been investigated in the last decade. Similar to usual pancreatic ductal adenocarcinomas (PDACs), IPMNs often harbor *KRAS* mutations (56%-66%), while *GNAS* mutations at codon 201 are almost exclusively observed in pancreatic IPMNs (48%-79%) among pancreatic neoplasms [5,6]. In one study, *GNAS* mutations were more commonly detected in gastric (45/97; 46%) and intestinal types (23/56; 59%) than in oncocytic (1/8; 14%) and pancreatobiliary types (3/11; 27%) [7]. Although the incidence of *GNAS* mutations varies among histologic types, *GNAS* is currently regarded as the most common driver gene of pancreatic IPMNs.

We experienced a couple of cases of PB-IPMNs that lacked cystic dilatation of the lesional ducts, the imaging appearance atypical for IPMNs. These cases also showed purely pancreatobiliary morphology, higher grades of cytological atypia, and more complex intraductal papillary architecture than did those expected in pancreatic IPMNs. This prompted us to hypothesize that some cases of PB-IPMNs may be more similar biologically to PDACs. Therefore, we systematically compared the clinicopathologic and genetic features between PB-IPMNs and conventional PDACs.

2. Materials and methods

2.1. Case selection

This study was approved by the Ethics Committee at Kobe University Graduate School of Medicine (No. 180223). The study consisted of 29 consecutive patients with PB-IPMN with or without associated invasive cancer who underwent pancreatic resection at Kobe University Hospital between 2001 and 2018 and 2 additional patients with PB-IPMN surgically treated at an affiliated hospital. The control group comprised 24 consecutive patients with PDAC who had surgical resection at our institute without neoadjuvant chemotherapy between 2014 and 2015.

According to pancreatic tissue examination standards at our institute, the resected pancreas was entirely submitted for microscopic evaluation after gross examination. Diagnosis and histologic classification of PB-IPMNs were established according to criteria proposed [3,8]. All PB-IPMNs examined in the present study had a grossly visible intraductal mass histologically consisting of mucin-producing cells. Immunohistochemistry for mucin core proteins (MUCs) was also used to characterize the tumor-cell phenotype. Degrees of dysplasia were classified into low and high grades [1]. In cases of IPMNs with multiple histologic types, the predominant type was used for classification.

PB-IPMNs were separated into monotypic and polytypic cases based on microscopic features, because atypical cases we experienced showed purely pancreatobiliary-type high-grade morphology. Polytypic cases were defined as PB-IPMNs that had minor components (comprising <30% of the whole tumor) of low-grade dysplasia and/or areas of other histologic types (eg, gastric, intestinal, or oncocytic types). PB-IPMNs consisting of entirely (100%) high-grade neoplastic cells with pancreatobiliary morphology were called monotypic tumors. All PB-IPMNs collected could be separated into either group. Two investigators (T. S. and Y. Z.) reviewed all histology slides together using a multiheader microscope and classified the cases into 2 groups without reference to clinical and imaging features.

2.2. Clinicopathologic features

Electronically stored clinical records were reviewed for age, sex, serum concentrations of tumor markers, tumor location, and postoperative prognosis. Reports and images of endoscopic examinations were also reviewed for dilatation of ampullary orifice and mucin secretion from the orifice. The pathologic parameters examined were tumor size, invasive malignancy, pathologic T stage (according to the eighth edition of Union for International Cancer Control classification), microscopic lymphovascular invasion, perineural infiltration, and lymph node metastasis. PB-IPMNs were classified into the main duct, branch duct, and mixed types based on imaging and histologic findings.

2.3. Imaging analysis

Imaging features by computed tomography (CT) were compared between monotypic and polytypic PB-IPMNs, particularly in terms of nodule size, duct dilatation (>5 mm in diameter) in the upstream or downstream ducts, and cysts in the

background pancreas. In addition, cases were classified into 3 groups based on the overall CT imaging appearance (Fig. 1). Type A was defined as cystic or fusiform dilation of pancreatic ducts with or without a mural nodule. Type B showed a solid mass without duct dilation. Type C presented a solid tumor with dilatation of the upstream pancreatic duct. Images obtained by other modalities, such as magnetic resonance imaging, were not used because they were not available in all cases.

2.4. Immunohistochemistry

Immunostaining was performed on Benchmark XT (Ventana Medical Systems, Tucson, AZ) or Bond Max autostainer (Leica Microsystems, Wetzlar Germany) according to the manufacturer's protocols. All cases were stained for MUC1, MUC2, MUC5AC, MUC6, p53, p16, and SMAD4. Immunostaining for SMAD4, p53, and p16 correlates with genetic alterations [9]. Deparaffinized sections were heat treated and incubated with primary antibodies. The antibodies used were as follows: MUC1 (clone Ma695; dilution 1:100; Leica Microsystems), MUC2 (clone Ccp58; dilution 1:100; Leica Microsystems), MUC5AC (clone CLH2; dilution 1:100; Leica Microsystems), MUC6 (clone CLH5; dilution 1:100; Leica Microsystems), SMAD4 (clone B-8; dilution 1:100; Santa Cruz Biotechnology, Santa Cruz, CA), p53 (clone DO-7; dilution 1:300; Leica Microsystems), and p16 (clone E6H4; ready-to-use; Roche Tissue Diagnostics, Tucson, AZ).

Immunohistochemical results were analyzed in intraductal components. As for MUCs, expression levels were evaluated semiquantitatively according to the percentage of positive cells: 0 (negative); 1+ (focal), 1% to 5%; 2+ (moderate), 6% to 50%; 3+ (diffuse), more than 50%. Cases with 2+ and 3+ expression levels were defined as positive for MUCs. A diffuse nuclear staining was defined as a positive p53 result, indicating a

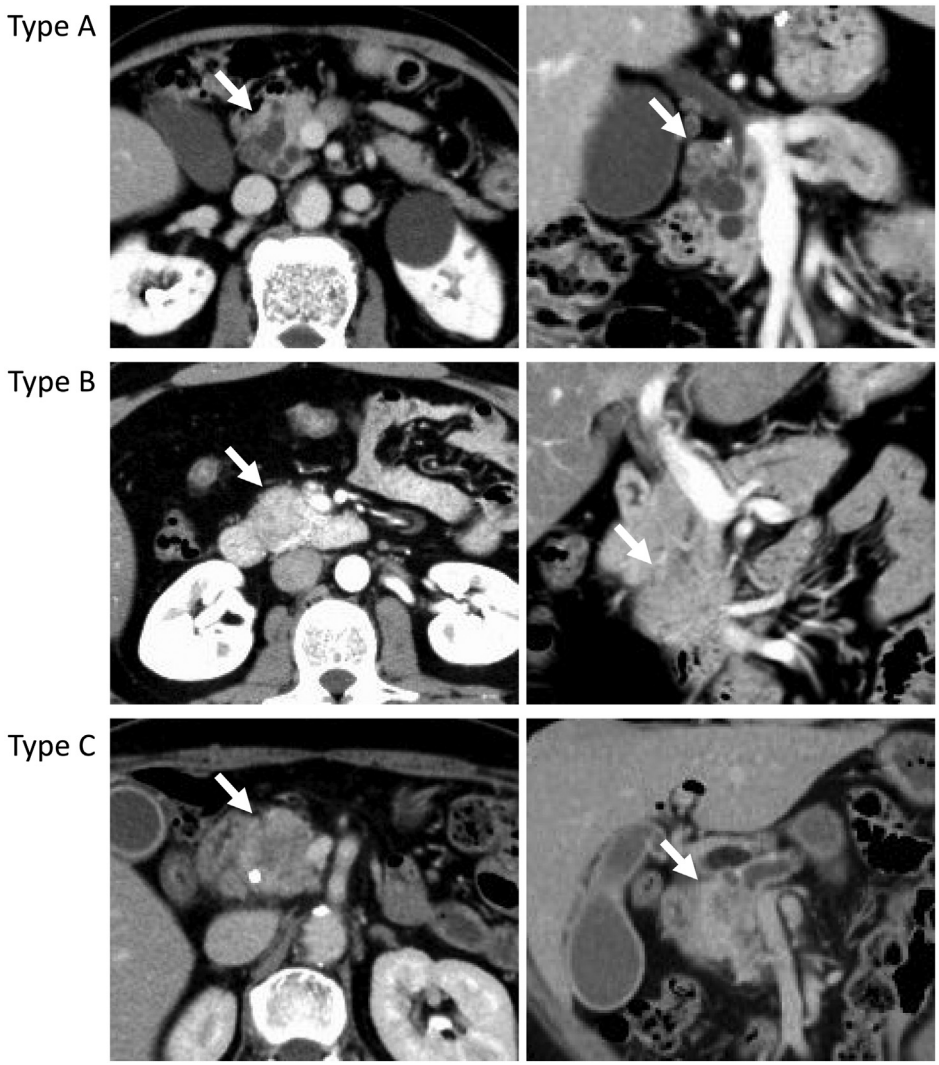

Fig. 1 Three types of PB-IPMNs by imaging. Type A was characterized by duct dilatation and intraductal mass lesion (arrows). Type B had a solid mass lesion without upstream duct dilatation (arrows). Type C was defined as a solid mass lesion with upstream duct dilatation (arrows). Axial (left) and coronal (right) CT images.

Table 1 Comparison of clinical features [a]

	Polytypic PB-IPMNs (n = 19)	Monotypic PB-IPMNs (n = 12)	PDACs (n = 24)	P [b]
Age (y)	73 [60–86]	73 [60–86]	67 [51–84]	.113
Female sex	10 (53%)	8 (67%)	9 (37%)	.238
Tumor markers (ng/mL)				
CA19-9 *	11.0 [1.0–294]	18.5 [1.0–331]	70.5 [1–30 680]	.024
CEA	2.5 [1.3–9.0]	2.2 [0.7–5.4]	2.75 [0.4–45.6]	.523
Tumor location				
Head	13 (68%)	8 (67%)	14 (58%)	.523
Body or tail	6 (32%)	4 (33%)	10 (42%)	
Dilatation of ampullary orifice *,**	14 (74%)	0	1 (4%)	<.001
Mucin secretion *,**	17 (89%)	2 (17%)	2 (8%)	<.001

[a] Continuous variables were shown as a median [range].
[b] P value was calculated in 3-group comparison, and $P < .01$ was considered to be significant (see the Materials and methods section).
* $P < .01$ between polytypic PB-IPMNs and PDACs.
** $P < .01$ between polytypic PB-IPMNs and monotypic PB-IPMNs.
*** $P < .01$ in monotypic PB-IPMNs versus PDACs.

mutation in p53. Completely negative nuclear staining of p16 was defined as loss of expression, indicating a mutation in *CDKN2A*. Cases with no detectable cytoplasmic or nuclear SMAD4 protein were scored as negative for SMAD4.

2.5. Gene mutation analysis

Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue blocks. Areas consisting predominantly of tumor cells were selected for DNA extraction under a microscope. DNA extraction was performed with the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA content was determined with a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Mutations in *KRAS* exon 2 (codons 12 and 13) were examined extensively by a droplet digital polymerase chain reaction protocol using the QX200 Droplet Digital PCR platform (BioRad, Pleasanton, CA) according to a previous report [10]. This approach reveals *KRAS* mutations in exon 2 (G12A, G12C, G12D, G12R, G12S, G12V, or G13D) but does not specify the type of mutation. In our preliminary study to optimize this assay, the sum of the *KRAS* mutation values (%) in 3 samples of unremarkable pancreatic tissue (background parenchyma of benign pancreatic tumors) was 0.74% ± 0.38% [11]. Therefore, the mean plus 3 SDs [12], which corresponds to more than 1.88% *KRAS* mutations, was considered significant.

GNAS mutations at exon 8 (codon 201) were analyzed by Sanger sequencing according to a previous report [13]. Sequences of primers used for gene amplification were as follows: 5′-ACTGTTTCGGTTGGCTTTGGTGA-3′ and 5′-AGGGACTGGGGTGAATGTCAAGA-3′.

Table 2 Comparison of CT findings [a]

	Polytypic PB-IPMNs (n = 19)	Monotypic PB-IPMNs (n = 12)	P [b]
Nodule size (mm)	9.4 [0–27.0]	19.4 [7.0–42.2]	.006
Upstream duct dilatation	12 (63%)	10 (83%)	.228
Downstream duct dilatation	13 (68%)	2 (17%)	.005
Background multiple cysts	10 (53%)	1 (8%)	.020
Overall appearance			<.001
Type A	18 (95%)	1 (8%)	
Type B	1 (5%)	2 (17%)	
Type C	0	9 (75%)	
Imaging diagnosis			.022
IPMN with or without invasive cancer	18 (95%)	7 (58%)	
Conventional PDAC	0	4 (33%)	
Others	1 (5%)	1 (8%)	
Localization			<.001
Main duct type	1 (6%)	8 (67%)	
Branch duct type	6 (33%)	3 (25%)	
Mixed type	11 (61%)	1 (8%)	

[a] Continuous variables were shown as median [range].
[b] $P < .01$ was considered to be significant (see the Materials and methods section).

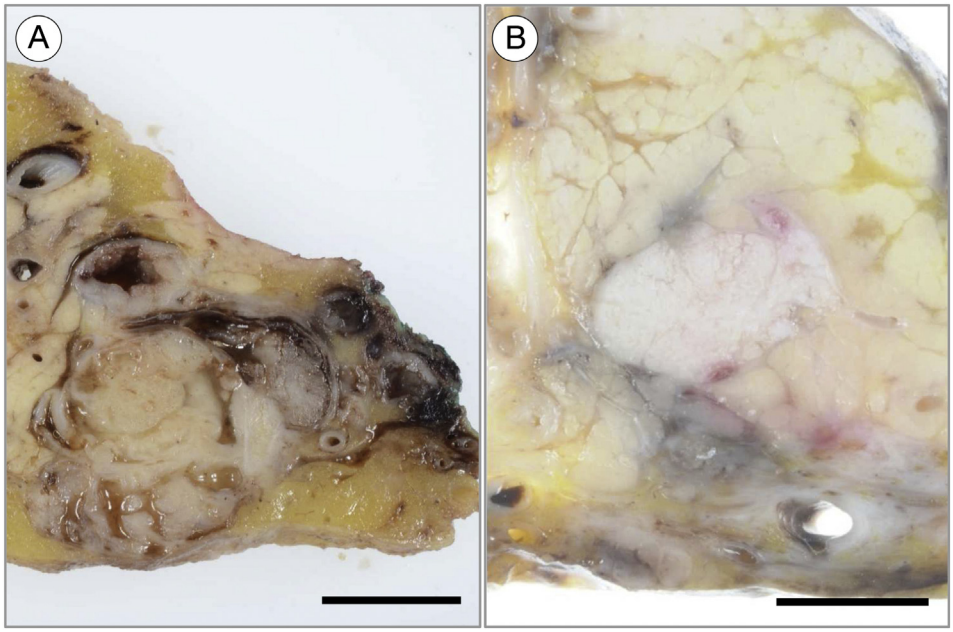

Fig. 2 Gross findings of PB-IPMNs. A, A polytypic PB-IPMN case had a papillary tumor in the dilated ducts. B, A monotypic case of PB-IPMN had an intraductal solid mass packed in the duct.

2.6. Statistical analysis

Continuous variables not showing a bell-shaped distribution were assessed using an unpaired *t* test, Mann-Whitney *U* test, or Kruskal-Wallis test. Three-group analysis was conducted first. When a significant difference was observed, 2-group subanalyses were performed to identify which 2 groups had significant differences. Recurrence-free survival was calculated using the Kaplan-Meier method, and the log-rank test was used to compare survival curves. JMP 14 (SAS Institute, Cary, NC) was used for statistical analyses. A probability of $P < .05$ was considered to be significant for comparisons of one or 2 parameters, and the cutoff value was adjusted to $P < .01$ based on the Bonferroni correction model for the comparison of clinicopathologic features because approximately 5 clinical, imaging, pathologic, or immunohistochemical parameters each were analyzed (0.05/5 parameters = 0.01).

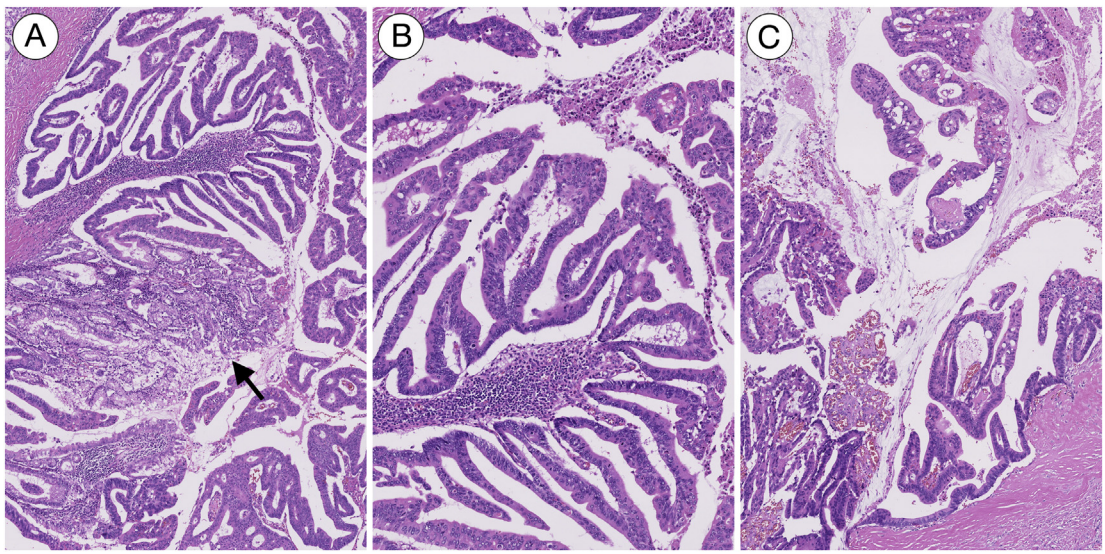

Fig. 3 Microscopy findings of polytypic PB-IPMNs. A, The intraductal papillary tumor was focally associated with a gastric-type component with low-grade dysplasia (arrow). B, Tumor cells were arranged in a high-papillary fashion with thin fibrous cores. C, The dilated duct contained accumulated mucus.

3. Results

3.1. Clinical features

Of the 31 cases of PB-IPMNs, 16 were associated with minor gastric-type components with low-grade dysplasia, and 3 other cases had oncocytic-type elements. Therefore, these 19 cases (61%) were classified as polytypic PB-IPNBs, and the remaining 12 (39%) with pure high-grade pancreatobiliary morphology were categorized as monotypic cases. As shown in Table 1, no significant difference was observed in terms of age, sex, tumor markers, and tumor location among polytypic PB-IPMNs, monotypic PB-IPMNs, and PDACs. In contrast, endoscopic findings of the ampulla of Vater seemed to significantly differ with dilatation of the ampullary orifice and mucin secretion observed in 14 (74%) and 17 (89%) of 19 polytypic PB-IPMNs, respectively, but these observations were markedly less common in monotypic PB-IPMNs (0% and 17%) and PDACs (4% and 8%; both $P < .001$).

3.2. CT findings

The size of solid or papillary nodules was significantly larger in monotypic PB-IPMNs than in polytypic cases ($P = .006$; Table 2). Polytypic PB-IPMNs also frequently presented dilatation of the downstream pancreatic duct (68%) and multiple cysts in the background pancreas (53%), but they were less common in monotypic cases (17% and 8%, respectively). All but one case of polytypic IPNB showed an overall type A imaging appearance; therefore, it was correctly diagnosed as an IPMN before surgery. In contrast, all but one case of monotypic PB-IPMN had features of type B or C. IPMNs were suspected only in 7 (58%) of 12 polytypic cases. One case of

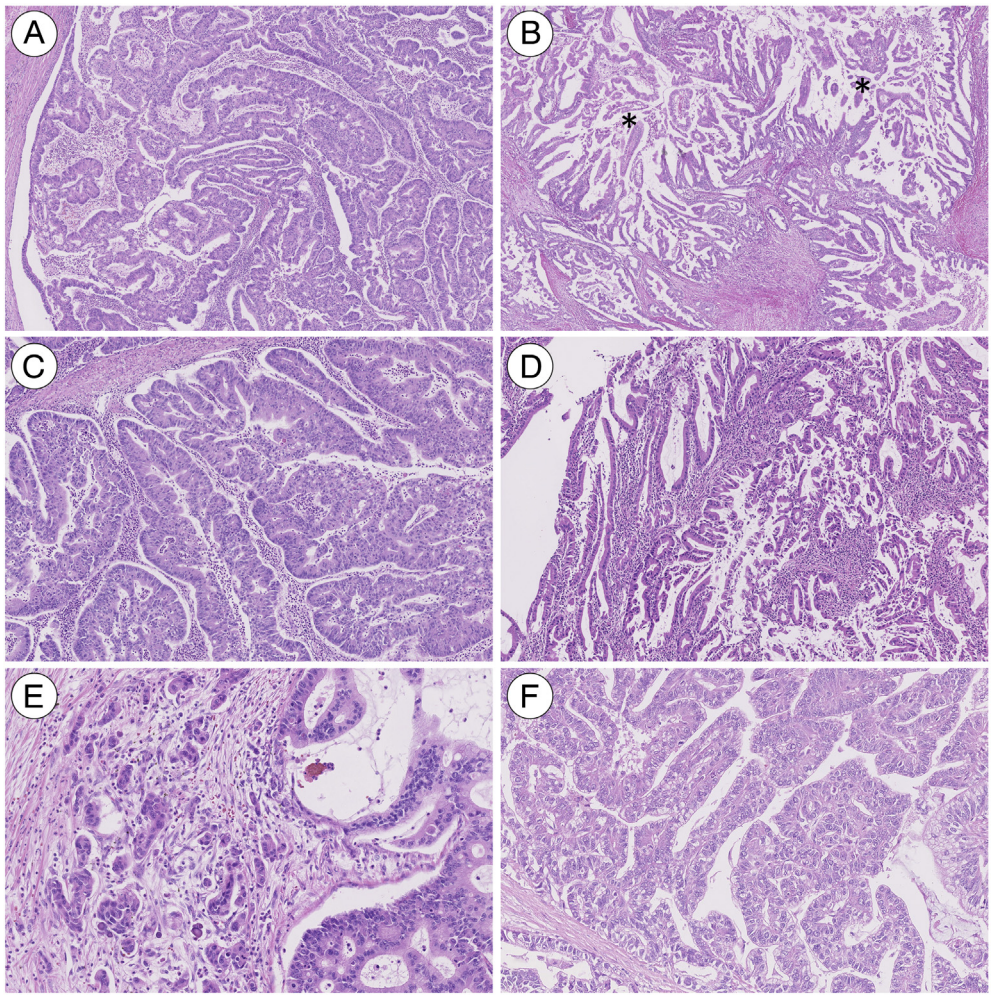

Fig. 4 Microscopy findings of monotypic PB-IPMNs. A, The duct was packed with an intraductal tumor showing a complex papillary architecture. B, Intraductal papillary neoplasm (*) and invasive papillary carcinoma were intermingled without an obvious border in between. C, Tumor cells were arranged in a complex papillary and micropapillary architecture. D, The stroma of the intraductal component was broad and inflamed. E, An invasive cancer was observed in the duct wall. F, Tumor cells had moderately pleomorphic nuclei and distorted cellular polarity consistent with high-grade dysplasia. Some foci of the intraductal components were suggestive of, but not conclusive for, stromal invasion.

polytypic PB-IPMN was suspected to be a neuroendocrine tumor, and one case of monotypic PB-IPMN was diagnosed as an ampullary cancer by imaging.

3.3. Pathologic features

All polytypic cases of PB-IPMNs had papillary tumors inside the mucus-containing dilated ducts by gross examination (Fig. 2A). In contrast, cystic duct dilatation, mucus accumulation, and papillary architecture were markedly less obvious in monotypic cases. A solid mass packed in the duct was the most common gross appearance (Fig. 2B). Two cases also showed an ill-defined mass lesion with a solid cut surface in which an intraductal tumor was vaguely identified.

Histologically, polytypic PB-IPMNs consisted of neoplastic cells arranged in a high-papillary fashion along thin fibrovascular cores. The cells had moderately pleomorphic nuclei and eosinophilic cytoplasm (Fig. 3). Cribriform or necrotic foci were focally observed. The ducts were dilated with accumulated extracellular mucus. The boundary among different components (lower-grade lesions or other histologic types) was relatively abrupt. Intraductal growth patterns of monotypic PB-IPMNs were more complex with variable degrees of tubular and cribriform components or irregular papillary branching identified (Fig. 4). Monotypic PB-IPMNs had broader fibrous stroma, more inflammation, and more conspicuous necrotic changes than polytypic cases. Some foci of complex papillary growth in the intraductal components were suggestive of early stromal invasion. Nevertheless, noninvasive intraductal papillary tumors were more widely observed in all cases by definition.

All invasive cancers that developed in a background of either type of PB-IPMN were ductal adenocarcinoma without components of mucinous carcinoma. In most cases, the invasive component was identified in the duct wall, separately from the noninvasive intraductal element (Fig. 4E), but 2 monotypic cases that grossly showed an ill-defined solid mass were characterized by randomly intermingled intraductal and infiltrative components with less obvious separation in between (Fig. 4B). However, the presence of a noninvasive intraductal papillary component met the criteria of IPMN diagnosis, and the appearance differed from PDACs with a cystic papillary growth pattern, in which cystic papillary glands represent an infiltrating cancer [4,14]. Retrospective radiopathologic correlation studies revealed that the main duct type without obvious branch duct involvement was most common in monotypic PB-IPMNs (67%), and the mixed type was most frequent in polytypic cases (61%; Table 2).

Monotypic PB-IPMNs had more aggressive pathologic features (Table 3). All cases of monotypic PB-IPMNs were associated with invasive cancers, whereas malignant transformation was confirmed in 7 (37%) of 19 polytypic cases ($P < .001$). More than a half of monotypic PB-IPMNs had lymph node metastasis, lymphovascular invasion, and

Table 3 Comparison of pathologic features [a]

	Polytypic PB-IPMNs (n = 19)	Monotypic PB-IPMNs (n = 12)	PDACs (n = 24)	P [b]
Tumor size (mm)	38 [13–93]	28 [16–60]	25 [14–62]	.098
Invasive cancer *,**	7 (37%)	12 (100%)	24 (100%)	<.001
pT classification *				<.001
pT0	9 (47%)	0	0	
pT1	4 (21%)	5 (42%)	6 (25%)	
pT2	4 (21%)	3 (25%)	15 (63%)	
pT3	2 (11%)	4 (33%)	3 (13%)	
pT4	0	0	0	
Lymph node metastasis **	1 (14%) [c]	6 (50%)	19 (80%)	.006
Lymphatic invasion	1 (14%) [c]	8 (67%)	12 (50%)	.087
Venous invasion **	2 (29%) [c]	5 (42%)	20 (83%)	.006
Perineural invasion **,***	1 (14%) [c]	7 (58%)	23 (96%)	<.001
Immunostaining				
MUC1 **	12 (63%)	11 (92%)	24 (100%)	.002
MUC2	4 (21%)	2 (17%)	0	.069
MUC5AC **	19 (100%)	11 (92%)	14 (59%)	.002
MUC6 **,***	13 (68%)	4 (33%)	0	<.001
p53	1 (5%)	2 (17%)	8 (33%)	.070
Loss of p16	10 (53%)	7 (58%)	18 (75%)	.290
Loss of SMAD4 **	2 (11%)	5 (42%)	15 (63%)	.003

[a] Continuous variables were shown as a median [range].
[b] P value was calculated in 3-group comparison, and $P < .01$ was considered to be significant (see the Materials and methods section).
[c] Only 7 cases with invasive malignancy examined
* $P < .01$ between polytypic PB-IPMNs and monotypic PB-IPMNs.
** $P < .01$ between polytypic PB-IPMNs and PDACs.
*** $P < .01$ in monotypic PB-IPMNs versus PDACs.

Table 4 Frequency of gene mutations

	Polytypic PB-IPMNs (n = 19)	Monotypic PB-IPMNs (n = 12)	PDACs (n = 24)	P [a]
KRAS mutation *,**	12 (63%)	10 (83%)	24 (100%)	.005
GNAS mutation *,***	6 (32%)	0	0	.002

[a] P value was calculated in 3-group comparison, and $P < .05$ was considered to be significant.
* $P < .01$ between polytypic PB-IPMNs and PDACs.
** $P < .01$ in monotypic PB-IPMNs versus PDACs.
*** $P < .01$ between polytypic PB-IPMNs and monotypic PB-IPMNs.

perineural infiltration. The tumor cell phenotype of polytypic PB-IPMNs was distinct from that of PDACs by immunohistochemistry, particularly for the expression of MUC1, MUC5AC, MUC6, and SMAD4, but the difference was markedly less obvious between monotypic PB-IPMNs and PDACs where only the expression of MUC6 differed between the 2 groups.

3.4. Mutations in *KRAS* and *GNAS*

Sequencing of *KRAS* and *GNAS* was successful in all cases. *KRAS* mutations were detected in 12 (63%) of 19 polytypic PB-IPMNs, 10 (83%) of 12 monotypic PB-IPMNs, and 24 (100%) of 24 PDACs (Table 4). *KRAS* mutations were less frequent in PB-IPMNs than in PDACs. *GNAS* R201H mutations were observed in 6 (32%) of 19 polytypic PB-IPMNs, whereas *GNAS* was wild type in all monotypic PB-IPMNs and PDACs ($P = .002$).

3.5. Postoperative prognosis

The median follow-up period was 32 months for polytypic PB-IPMNs, 21 months for monotypic PB-IPMNs, and 27 months for PDACs. As shown in Fig. 5, patients with polytypic PB-IPMN had a better recurrence-free survival than did those with monotypic IPMN or PDAC. Recurrence-free survival rates at 1 and 3 years after surgery were 94% and 89% for polytypic PB-IPMNs, 50% and 50% for monotypic PB-IPMNs, and 49% and 37% for PDACs, respectively. The survival curves of monotypic PB-IPMNs and PDACs were not significantly different. The median recurrence-free survival was 9 months for monotypic PB-IPMNs and 12 months for PDACs. No significant difference of prognosis was observed among pT1, pT2, and pT3 cancers in the monotypic PB-IPMN group ($P = .980$).

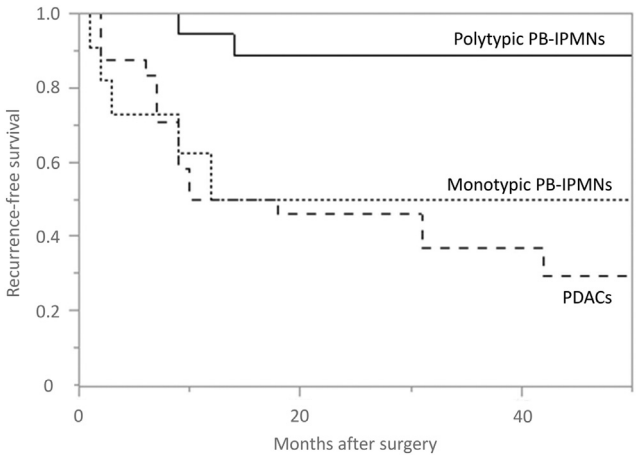

Fig. 5 Comparison of the prognosis among polytypic PB-IPMNs, monotypic PB-IPMNs, and PDACs. The recurrence-free survival curve of patients with monotypic PB-IPMN was similar to that of patients with PDAC and significantly worse than that of patients with polytypic PB-IPMN. $P = .006$ in polytypic versus monotypic PB-IPMNs; $P = .001$ in polytypic PB-IPMNs versus PDACs.

4. Discussion

Our results can be summarized as follows: (1) approximately one-third of PB-IPMNs consisted of purely high-grade pancreatobiliary type cells, and they had imaging and/or pathologic features that were atypical for IPMNs. (2) In monotypic cases, dilatation of the ampullary orifice and mucin overproduction were uncommon. (3) Most cases of monotypic PB-IPMNs lacked cystic dilatation of the lesional ducts by imaging; therefore, one-third of the cases were diagnosed as PDACs. (4) Histologically, intraductal components of monotypic PB-IPMNs were characterized by complex papillary growth with cribriform glands, necrosis, irregular micropapillary branching, and broad fibrous stroma, and always associated with invasive malignancy. (5) No *GNAS* mutations were observed in monotypic PB-IPMNs. (6) The prognosis of patients with monotypic PB-IPMN was markedly worse than that of patients with polytypic PB-IPMN and was almost identical to that of patients with PDAC.

Monotypic PB-IPMNs meet the diagnostic criteria for IPMNs because of the presence of a grossly visible, noninvasive intraductal papillary component. However, *GNAS* mutations were not observed in monotypic PB-IPMNs. The presence of *GNAS* mutations is currently regarded as genetic evidence of IPMN origin [15]. Small cystic changes with *GNAS* mutations are called incipient IPMNs [16]. The presence of *GNAS* mutations in PDACs suggests the possibility of malignant transformation of IPMNs [17]. *SMAD4* mutations are also known to be more common in PDACs than in any types of IPMNs [18]. SMAD4 expression was lost in 42% of monotypic PB-IPMNs and in 63% of PDACs, but in

only 11% of polytypic PB-IPMNs. Furthermore, although pancreatic IPMNs are usually diagnosed by imaging features, it was possible in only 58% of monotypic cases because the absence of cystic dilatation of lesional ducts exhibited a solid appearance on imaging. A potential interpretation of the differences between monotypic and polytypic PB-IPMNs and similarities between monotypic PB-IPMNs and PDACs is that monotypic PB-IPMNs are not true IPMNs but a papillary variant of conventional PDACs.

In many other organs, there is a pathologic category of papillary carcinoma such as papillary adenocarcinoma in the stomach and papillary squamous cell carcinoma in the head and neck. We recently proposed morphological separation criteria between papillary cholangiocarcinomas and intraductal papillary neoplasms of the bile duct (IPNBs) [19,20]. IPNBs meeting stringent histopathologic criteria differ from papillary cholangiocarcinomas in their location, immunohistochemical phenotype of tumor cells, and patients' prognosis [19,21]. In an exome-sequencing study, mutations in *APC* and *CTNNB1* were detected in 43% of IPNBs meeting the histologic criteria but were exceptional in papillary and nonpapillary cholangiocarcinomas [22]. In addition, the same standard can be applicable to gallbladder papillary neoplasm. Interestingly, mutations in *STK11*, the causative gene of Peutz Jegher syndrome, in addition to *APC* and *CTNNB1* were almost exclusively detected in intracholecystic papillary neoplasms [23], further confirming that papillary adenocarcinoma is pathologically and genetically distinct from IPNB/intracholecystic papillary neoplasm.

In contrast, the category of papillary adenocarcinoma does not exist in the pancreas. The present study raised the question whether some cases classified as monotypic PB-IPMNs may correspond to that group. The presence of a noninvasive papillary component indicates an IPMN origin. However, papillary adenocarcinomas in many other organs do not always arise from papillomas [24]. Invasive cancers also replace the epithelial layer (so-called cancerization) and may have papillary proliferations. The highly dysplastic cellular appearance observed in some monotypic PB-IPMNs may suggest this possibility. In addition, some cases had microscopic foci in the intraductal components suggestive of, but not conclusive for, stromal invasion, a finding commonly seen in papillary carcinomas in other organs. We defined polytypic PB-IPMNs as tumors with either low-grade or other histologic components. We provisionally used this standard to select PB-IPMNs with unquestionable features of IPMNs, but do not mean to suggest that all tumors outside these criteria are not typical for IPMNs.

This study had some limitations. First, because of the rarity of PB-IPMNs, the number of cases was small. Second, only 2 genes were examined. A global sequencing analysis is needed to determine whether monotypic PB-IPMNs are more genetically similar to IPMNs than to PDACs.

In conclusion, some cases of PB-IPMNs, particularly monotypic ones, showed a PDAC-like imaging appearance and more distorted histologic features than typically seen in IPMNs. Genetically, these cases lacked *GNAS* mutations. Future studies with large cohorts should be focused on this atypical category to determine whether these cases represent a papillary variant of PDACs rather than IPMNs.

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