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R0 resection for ductal pancreatic cancer—Japanese experience

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Abstract

Since 1981, the Japan Pancreas Society (JPS) and National Cancer Center have jointly maintained the nationwide pancreatic cancer registry. Currently, there are 28,655 cases registered, with 12,608 cases of histologically confirmed invasive pancreatic cancer. Since the last revision of the International Union Against Cancer (UICC) classification in 2002, the survival analysis of the patients registered from 2001 to 2004 became possible for the first time. Using this detailed database, the rational of R0 resection was investigated. From 2001 to 2004, 2617 cases of histologically confirmed invasive ductal carcinoma of the pancreas were registered. According to the UICC classification, R0 resection becomes problematic mostly in UICC stage IIa and IIb. Of 1039 patients who underwent pancreatectomy for the tumor in the head of the pancreas, 160 had UICC stage IIa disease, and 468 had UICC stage IIb disease. The relationship between the survival and the extent of disease, together with portal vein (PV) resection, plexus (PL) resection, were analyzed. PV, retroperitoneal, and PL infiltration had a significant impact on the accomplishment of R0 resection in univariate and multivariate analyses. There was no advantage of PV resection for both PV (−) and PV (+) disease among UICC stage IIa or IIb patients, suggesting no benefit of prophylactic PV resection. Similarly, survival depends on PL invasion but not on combined resection of PL. There was no survival benefit associated with the extent of lymph node dissection. Survival among patients treated in the 2000s, after the introduction of gemcitabine in Japan, is significantly better than that of patients treated in the 1980s and 1990s. Although survival after pancreatectomy depends on the extent of disease rather than the surgical procedures, the chronologic improvement in survival indicates that chemotherapy had a significant impact on survival; development of new treatment modalities is awaited.

Keywords: Pancreatic cancer; R0 resection; Registry; Extended resection

Why does pancreatic cancer recur? Cancer cells beyond the surgical margin survive and regrow. Is this a systemic disease? There are definitely cases cured by surgery alone. The definitions of the R classification by the International Union Against Cancer (UICC) [1] apply to all digestive system tumors that indicate the absence or presence of residual tumor after treatment described by the symbol R as follows: R0 = no residual tumor; R1 = microscopic residual tumor; R2 = macroscopic residual tumor; RX = presence of residual tumor cannot be assessed.

After the regional pancreatectomy was reported by Fortner [2], Japanese surgeons made great efforts to achieve real R0 resection [3–9], especially in the 1990s when the perioperative management was improved to decrease the mortality and morbidity of pancreatic surgery. The Japan Pancreas Society (JPS) and National Cancer Center have jointly maintained the nationwide pancreatic cancer registry [10]. There are currently 28,655 cases registered, and 12,608 cases are histologically confirmed invasive pancreatic cancer. Since the last revision of the UICC classification in 2002 [1], the survival analysis of the patients registered from 2001 to 2004 became possible for the first time. Using this detailed database, the rational for R0 resection was investigated.

Patients and Methods

Each patient newly treated during the period 2001–2004 in the leading hospitals in Japan was registered using online submission of FileMaker Pro 7 records. Of 5,430 patients

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registered during this period, 1,039 underwent pancreatectomy for invasive ductal carcinoma in the head of the pancreas. The clinicopathologic profile of each patient consists of more than 350 parameters. The extent of the tumor was registered according to both the JPS [11] and UICC [1] classifications. Conclusive UICC stage was calculated by tumor-node-metastasis (TNM) factors declared by the attending physician or surgeon. Conclusive T, N, and M factors are total findings of histopathologic, surgical, and radiologic examinations. As R0 resection becomes problematic mostly in UICC stage IIa (T3,N0,M0) and IIb (T1–3,N1,M0), patients with a lesser extent (tumor confined in the pancreas) or greater extent (tumor infiltrating to superior mesenteric artery and/or celiac axis and/or disseminated) of disease were excluded from the analysis. There were 160 patients with UICC stage IIa disease and 468 patients with UICC stage IIb disease. The relationship between R factor and each extent factor, including bile duct (CH), duodenal (DU), anterior serosal surface (S), retroperitoneal (RP), portal vein (PV), artery (A), peripancreatic plexus (PL), other organ involvement (OO), and nodal involvement (N), was analyzed, together with treatment factors, such as combined resection of portal vein (PVR), peripancreatic plexus (PLR), and the level of dissection (D). The relationship between the survival rate and PVR and/or PLR stratified with the tumor infiltration was analyzed using the actuarial method. The mean follow-up period is 11.8 months (median 9, range 0 to 46 months) for these 628 patients. Chi-square test was used for numerical data. Logistic regression analysis was used for multivariate analysis. The survival rate was examined by generalized Wilcoxon test. $P < .05$ was considered statistically significant.

Results

The histologic subtypes of the tumor did not correlate with the success rate of R0 resection, although R2 resection was observed only in tubular and adenosquamous carcinoma (Table 1). Univariate analysis of the tumor extent factors revealed that TS, DU, OO, and N did not affect R level. On the other hand, S, RP, CH, PV, A, and PL had a significant impact on R level. It should be noted that "A" factor in these stages means arteries other than the supramesenteric artery (SMA) and celiac axis (CA), ie, common hepatic artery or splenic artery. In terms of treatment factor, D, PVR, and PLR did not have a significant impact on R level. Multivariate analysis of these factors by logistic regression analysis shows that RP, PV, and PL had the strongest impact on the R level, followed by PVR and PLR, which had negative correlation coefficients (Table 2). The survival rate stratified with PVR is shown in Fig. 1. The survival rate of patients who had no PV infiltration after PVR was significantly worse than that of patients who did not undergo PVR (Fig. 1A). Among patients who had PV infiltration, PVR did not affect survival (Fig. 1B). The median survival time (MST) of the patients who underwent PVR without conclusive evidence of PV infiltration was 14.3 months, which was very close to that of the patients who underwent PVR because of PV infiltration. Similarly, PLR in PL (−) and PL (+) patients did not improve the survival in each stratum (Fig. 2A and B). The MST of patients with PLR without conclusive evidence of PL infiltration was 5 months longer than that of the patients without PLR in the same stratum, but the difference was not statistically significant (Fig. 2A). The MSTs of patients with and without PLR in the PL (+) stratum were 14.5 and 14.2 months, respectively, with no statistical difference (Fig. 2B).

Fig. 3 shows the survival rate according to the R levels for UICC stage IIa and IIb disease. If R0 operation was performed, survival of the patients was significantly better than that of the patients with R1 operation. The difference between R1 and R2 was not statistically significant due to the small number of patients with R2 operation.

Finally, the survival rate of the patients treated during the period 2001–2004 was compared with that of the patients treated in the 1980s and 1990s. As shown in Fig. 4, the survival rate was significantly improved in the third period, whereas the improvement was not significant between the first and second periods.

Comments

Cancer staging is critically important not only to define the extent of disease and to determine the treatment strategies, but also to compare the treatment results between institutions and countries. The UICC classification [1], as well as the American classification [12], is determined by T factor strongly giving weight to the infiltration to SMA and CA together with simple N and M factors. UICC T4 reflects the difficulty of resection of the tumor in this extent [13]. On the other hand, the JPS classification [11] is similar to the previous version of the UICC classification [14] but is based on the survival outcome of every combination of TNM factors. JPS regards T factor as a function consisting of the factors TS, S, RP, CH, DU, PV, A, PL, and OO, so that more detailed analysis is possible. These factors also can be applied to UICC T factors. In this analysis, patients with UICC stage IIa and IIb determined by the attending physicians were selected to determine the factors that might have an impact on the accomplishment of R0 resection. Even in the same stage, the infiltration to the PV, RP, and peripancreatic PL significantly affected R level by univariate and multivariate analysis. Furthermore, combined resection of PV and PL did not improve or worsen survival regardless of infiltration, suggesting that prophylactic resection of PV and PL is not necessary and that combined resection is rational if necessary.

Preoperative evaluation of PV, PL, and arterial infiltration is difficult even in the era of high-resolution imaging [15]. It strongly depends on the experience of radiologists and surgeons to judge whether the tumor is resectable or not. Even if PV infiltration is strongly suspected preoperatively, combined resection of the PV is not always necessary. On the other hand, there is a dilemma that no histologic confirmation can be obtained without combined resection. So, the registry requires the conclusive findings of each factor based on any of the radiologic, surgical, and histologic findings.

As shown in Fig. 3, the survival of patients with UICC stage IIa and IIb disease, the R0 resection has a positive impact on outcome. The number of patients with UICC stage IIb (N+) was not different in the R0 and R1 groups

Table 1
Univariate analysis of each factor that may affect R levels for UICC stage IIa and IIb disease

	R0	R1	R2	Total	*P*
Papillary	8 (8.0%)	2 (2.0%)	0 (.0%)	10 (10.0%)	NS
Adeno	18 (85.7%)	3 (14.3%)	0 (.0%)	21 (10.0%)	
Well differentiated	131 (78.4%)	30 (18.0%)	6 (3.6%)	167 (10.0%)	
Moderately differentiated	257 (78.1%)	63 (19.1%)	9 (2.7%)	329 (10.0%)	
Poorly differentiated	49 (77.8%)	13 (2.6%)	1 (1.6%)	63 (10.0%)	
Adenosquamous	12 (8.0%)	2 (13.3%)	1 (6.7%)	15 (10.0%)	
Mucinous	7 (87.5%)	1 (12.5%)	0 (.0%)	8 (10.0%)	
Anaplastic	0 (.0%)	2 (10.0%)	0 (.0%)	2 (10.0%)	
Acinar cell carcinoma	3 (10.0%)	0 (.0%)	0 (.0%)	3 (10.0%)	
Undifferentiated	1 (10.0%)	0 (.0%)	0 (.0%)	1 (10.0%)	
TS1 (≤ 2.0 cm)	61 (88.4%)	8 (11.6%)	0 (.0%)	69 (10.0%)	NS
TS2 (2.1–4.0 cm)	331 (77.7%)	82 (19.2%)	13 (3.1%)	426 (10.0%)	
TS3 (4.1–6.0 cm)	80 (76.2%)	21 (2.0%)	4 (3.8%)	105 (10.0%)	
TS4 (> 6.1 cm)	12 (75.0%)	4 (25.0%)	0 (.0%)	16 (10.0%)	
S(−)	324 (81.8%)	65 (16.4%)	7 (1.8%)	396 (10.0%)	.005
S(+)	149 (71.3%)	50 (23.9%)	10 (4.8%)	209 (10.0%)	
RP(−)	230 (89.5%)	23 (8.9%)	4 (1.6%)	257 (10.0%)	<.001
RP(+)	246 (7.3%)	91 (26.0%)	13 (3.7%)	350 (10.0%)	
CH(−)	162 (85.3%)	27 (14.2%)	1 (.5%)	190 (10.0%)	.008
CH(+)	320 (75.5%)	88 (2.8%)	16 (3.8%)	424 (10.0%)	
DU(−)	197 (78.8%)	45 (18.0%)	8 (3.2%)	250 (10.0%)	NS
DU(+)	286 (78.6%)	69 (19.0%)	9 (2.5%)	364 (10.0%)	
PV(−)	334 (85.2%)	55 (14.0%)	3 (.8%)	392 (10.0%)	<.001
PV(+)	147 (67.4%)	57 (26.1%)	14 (6.4%)	218 (10.0%)	
A(−)	469 (79.4%)	108 (18.3%)	14 (2.4%)	591 (10.0%)	<.001
A(+)	9 (52.9%)	5 (29.4%)	3 (17.6%)	17 (10.0%)	
PL(−)	379 (85.7%)	60 (13.6%)	3 (.7%)	442 (10.0%)	<.001
PL(+)	84 (55.6%)	54 (35.8%)	13 (8.6%)	151 (10.0%)	
OO(−)	4 (5.0%)	2 (25.0%)	2 (25.0%)	8 (10.0%)	NS
OO(+)	5 (71.4%)	2 (28.6%)	0 (.0%)	7 (10.0%)	
N0	128 (82.6%)	23 (14.8%)	4 (2.6%)	155 (10.0%)	NS
N1	353 (77.8%)	89 (19.6%)	12 (2.6%)	454 (10.0%)	
D0	15 (83.3%)	3 (16.7%)	0 (.0%)	18 (10.0%)	NS
D1	8 (61.5%)	5 (38.5%)	0 (.0%)	13 (10.0%)	
D2	48 (68.6%)	20 (28.6%)	2 (2.9%)	70 (10.0%)	
D3	411 (8.4%)	85 (16.6%)	15 (2.9%)	511 (10.0%)	
PVR(−)	311 (79.3%)	67 (17.1%)	14 (3.6%)	392 (10.0%)	NS
PVR(+)	174 (77.0%)	49 (21.7%)	3 (1.3%)	226 (10.0%)	
PLR(−)	282 (77.3%)	72 (19.7%)	11 (3.0%)	365 (10.0%)	NS
PLR(+)	199 (8.2%)	43 (17.3%)	6 (2.4%)	248 (10.0%)	

NS = not significant; Adeno = adenocarcinoma without any description of differentiation; TS = tumor size; S = serosal surface infiltration; RP = retroperitoneal infiltration; CH= choledochal infiltration; DU = duodenal infiltration; PV = portal venous infiltration; A = arterial infiltration; PL = peripancreatic plexus infiltration; OO = other organ infiltration; PVR = portal vein resection; PLR = plexus resection.

Table 2
Logistic regression analysis of factors that may affect R levels for UICC stage IIa and IIb disease

Independent variables	Significance	Correlation coefficient
TS1: TS2: TS3: TS4	.8507	.0000
CH(−): CH(+)	.1092	.0315
S(−): S(+)	.1046	.0334
RP(−): RP(+)	.0001	.1535
PV(−): PV(+)	.0002	.1473
A(−): A(+) but not in SMA or CA	.7537	.0000
PL(−): PL(+)	.0000	.1941
N0: N1	.4863	.0000
D0: D1: D2: D3	.1449	−.0148
Combined resection of PV (PVR)	.0145	−.0835
Combined resection of PL (PLR)	.0249	−.0729

Dependent variable; R0 or more.

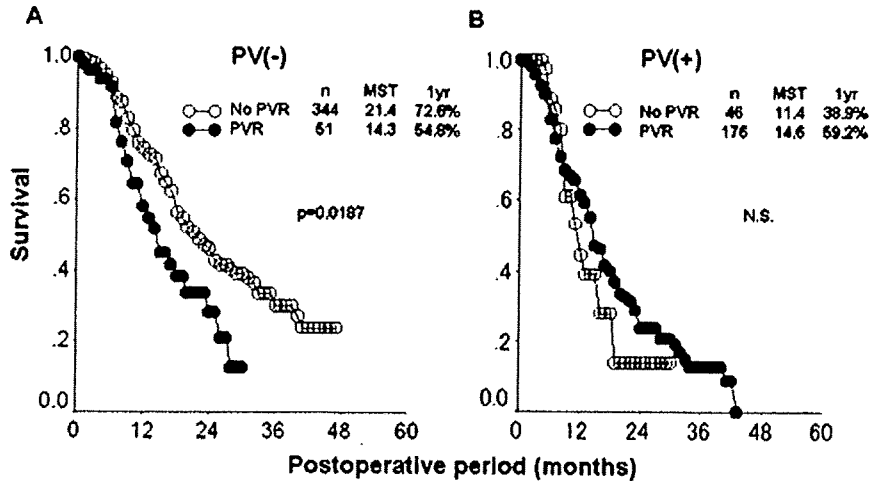

Fig. 1. Portal vein (PV) resection and cumulative survival of patients with International Union Against Cancer stage IIa and IIb. MST = median survival time.

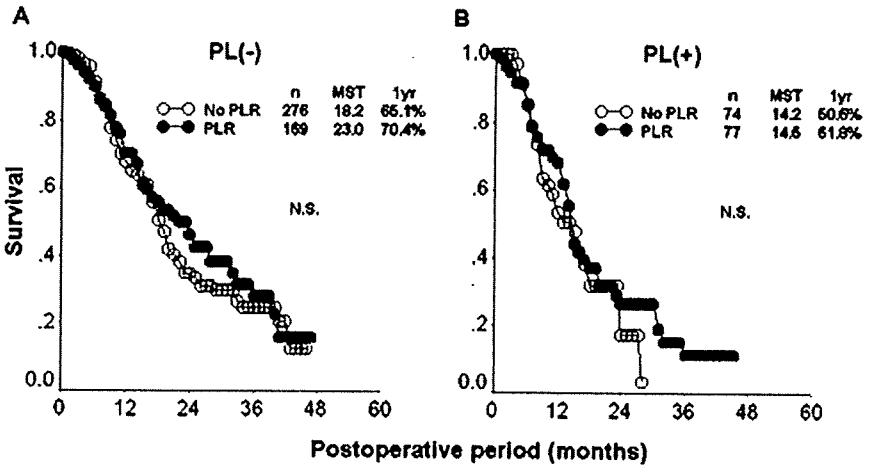

Fig. 2. Plexus resection and cumulative survival of patients with International Union Against Cancer stage IIa and IIb. MST = median survival time; PL = peripancreatic plexus infiltration; PLR = peripancreatic plexus resection.

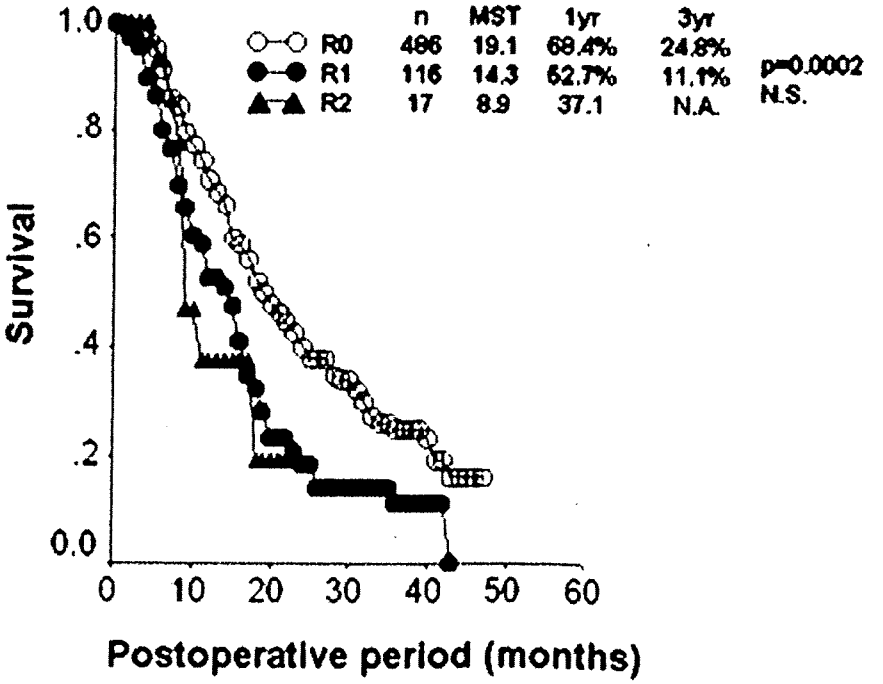

Fig. 3. R level and survival after pancreatectomy for International Union Against Cancer stage IIa and IIb disease. MST = median survival time.

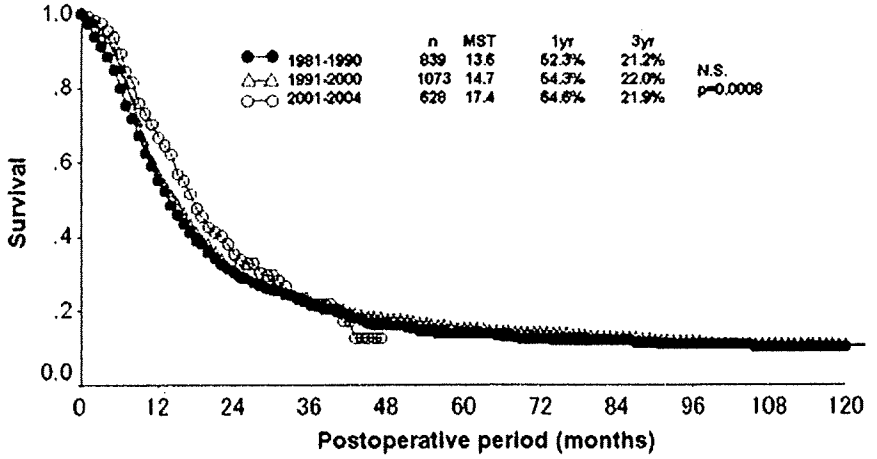

Fig. 4. Trend of survival after pancreatectomy for International Union Against Cancer stage IIa and IIb disease. MST = median survival time.

(data not shown), suggesting that lymph node status and the level of dissection had little impact on R level. It can be speculated that better survival was accomplished due to the lesser extent of disease rather than the aggressiveness of surgery.

Additionally, the trend of overall survival as shown in Fig. 4 suggests that there had been little improvement from 1980 to 1990, whereas significant improvement seems to have been achieved in the 2000s. In the 1980s, the mortality and morbidity of pancreatic surgery was still high. In the 1990s, the perioperative management was improved and radical operations, including combined resection and extended retroperitoneal dissection with or without intraoperative radiotherapy, were performed in many institutions in Japan. In April 2001, gemcitabine was clinically introduced in Japan and became widely used as adjuvant and therapeutic agent in a short period. Since the follow-up period is short for these newly registered patients, further correction of records and long-term follow-up is required. These chronologic changes indicate that the development of new treatment modalities combined with surgery is strongly awaited.

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RAPID COMMUNICATION

Elucidation of the relationship of BNIP3 expression to gemcitabine chemosensitivity and prognosis

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Abstract

AIM: To evaluate the significance of BNIP3 in the pathogenesis of pancreatic cancer, we analyzed the relationship between the expression of BNIP3 and survival rate of the patients with pancreatic cancer, or chemosensitivities in pancreatic cancer cell lines, particularly for gemcitabine, the first-line anti-tumor drug for pancreatic cancer.

METHODS: To compare the expression level of BNIP3 with the resistance to gemcitabine, eight pancreatic cancer cell lines were subjected to gemcitabine treatment and the quantitative real-time RT-PCR method was used to evaluate *BNIP3* expression. Immunohistochemical analysis was also performed using 22 pancreatic cancer specimens to study relationship between BNIP3 expression and survival rate.

RESULTS: Although no significantly positive association between *BNIP3* mRNA level and gemcitabine chemosensitivity was observed, pancreatic cancer cell lines that were sensitive to gemcitabine treatment tended to show high levels of *BNIP3* expression. The converse, an absence of *BNIP3* expression, was not correlated with gemcitabine resistance. We further compared the BNIP3 expression profiles of resected primary pancreatic cancer specimens with the prognosis of the patients, and found a tendency of favorable prognosis and low BNIP3 expression.

CONCLUSION: High levels of *BNIP3* expression cannot be used as one of the predicting factors for gemcitabine chemosensitivity, and some yet to be known factors will have to fill the gap for the accurate prediction of pancreatic cancer chemosensitivity to gemcitabine. However, BNIP3 expression may have an impact on prediction of prognosis of patients with pancreatic cancer.

Key words: BNIP3; Chemosensitivity; Gemcitabine; Pancreatic cancer; Prognosis

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INTRODUCTION

Pancreatic adenocarcinoma is a common cancer with an extremely poor prognosis around the world because of its aggressive invasive capacity, early metastasis, resistance to existing chemotherapeutic agents and radiation therapy, and lack of specific symptoms that help in finding patients at early stages for curative operation. To improve the horrible prognosis, we need to find novel approaches to both diagnosis and treatment that are more efficient than currently available techniques.

BNIP3, the hypoxia-inducible proapoptotic gene belonging to the *BCL2* family, was originally identified as the gene encoding a protein that interacts with adenovirus E1B 19-kDa protein[1]. The expression of BNIP3 is increased under hypoxic conditions by a transcription factor, hypoxia-inducible factor 1α (*HIF1α*)[2,3], and leads to cell death by two different pathways; (1) heterodimerization with the anti-apoptotic protein BCL2, and (2) opening the mitochondrial permeability transition pores by direct contact with its outer membrane. Thus, *BNIP3* is considered to be a key regulator

of hypoxia-induced cell death[4,5,6].

Hypoxia is a common phenomenon in solid tumors and has been proven to occur in pancreatic cancer, but *BNIP3* expression was shown to be decreased in pancreatic cancer compared with normal pancreas due to the hypermethylation of its promoter[7]. On the other hand, high expression of *BNIP3* in pancreatic cancer cell lines has been reported to be associated with the chemosensitivity to gemcitabine 2', 2'-difluorodeoxycitidin e. (Gemzar, Eli-Lilly, Indianapolis, IN), a novel pyrimidine nucleoside analogue[8]. It was expected that downregulation of proapoptotic *BNIP3* might contribute to chemo-resistance, survival and progression of pancreatic cancer in a hypoxic environment. In the present study, we analyzed the expression of *BNIP3* in several pancreatic cancer cell lines to explore the association with chemosensitivity in pancreatic cancer.

MATERIALS AND METHODS

Pancreatic cancer cell lines and cell culture

Two human pancreatic cancer cell lines (CFPAC1 and SUIT2) were obtained from Cancer Research UK Research Services (London, UK). PK-1, PK-8, PK-9, PK-45, and PK-59 were established at Tohoku University from patients with pancreatic cancer[9,10]. PANC-1 was obtained from Cell Resource Center for Biomedical Research, Institute for Development, Aging, and Cancer, Tohoku University (Sendai, Japan). All cells were maintained in RPMI 1640 containing 10% fetal bovine serum under an atmosphere of 5% CO_2 with humidity at 37℃.

MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay

To assess cell proliferation, the MTT test was used as described previously[11]. Cells were resuspended in fresh medium and seeded in 96-well plates at concentration of 5×10^3 cells/well. Cells were incubated for 24 h at 37℃, and then gemcitabine was added to each well at various concentrations. Each plate was incubated at 37℃ for 72 h. An aliquot of 10 μL of 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (2.5 mg/mL) was added to each well, and the plate was incubated at 37℃ for 2 h. The absorbance was measured at 560 nm using a microplate reader.

Quantitative real-time reverse transcription PCR

Total RNAs were extracted from the harvested cells using an RNeasy mini kit (QIAGEN, Tokyo, Japan) according to the supplier's instructions. Each purified RNA was dissolved in RNase-free water, and its concentration was measured by optical absorbance at an aliquot of 10 μg total RNA and Super Script II Reverse Transcriptase (Invitrogen, Carlsbad, CA) by the methods described previously[12]. The synthesized cDNA was used for a quantitative real-time PCR analysis using an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) following the manufacturer's instructions. Specific primers and common probe were designed by using the Primer Express software (Applied Biosystems), and their nucleotide sequences are: 5'-tggacggagtagctcc aagagc-3' (forward primer), 5'-agaagccctgttggtatcttgtg-3' (reverse primer), 5'-tctcactgtgacagtccacctcgc-3' (TaqMan probe). These primers were purchased from Nihon Gene Research Laboratories (Sendai, Japan). Expression of the β2-microglobulin (*B2M*) gene was monitored as an internal control, and the nucleotide sequences for the primers and the probe were described previously[13]. Amplifications were carried out in the reaction mixture in 25 μL containing 5 μL of cDNA samples and 12.5 μL of 2 × ABsolute QPCR ROX Mix (ABgene, Epsom, UK), and the final concentration of 0.2 μmol/L of each primer pair and 0.4 μmol/L of the probe were added in a program comprised of 2 min at 50℃, 15 min at 95℃, followed by 40 cycles consisting of 15 s at 95℃ and 1 min at 60℃. The expression ratio of *BNIP3/B2M* was calculated and used. Each experimental reaction was performed in triplicate.

Tissue samples and immunohistochemical analysis

Pancreatic cancer specimens were obtained from 22 patients between 1995 and 2004. All the patients had undergone surgery at Tohoku University Hospital. Stages were defined according to the general rules of the Japan Pancreas Society[14]. These patients underwent either pancreaticoduodenectomy or distal pancreatectomy with nodal dissection followed by similar chemotherapy including gemcitabine treatment. All of the patients' prognoses until 2005 were also determined. Twelve patients were male and 10 patients were female, and the mean age of operation was 64.0 years old. Specimens for immunohistochemistry were fixed in 10% formalin and embedded in paraffin for histological analysis. Research protocols for this study were approved by the Ethics Committee of the Tohoku University School of Medicine. Antigen retrieval was performed by boiling the slides in 10 mmol/L citrate buffer twice for 10 min. Peroxidase was quenched with a 3% H_2O_2 solution in 30% methanol. After an overnight incubation with mouse anti-BNIP3 antibody clone Ana 40 (Sigma-Aldrich, St. Louis, MO) at 4℃, slides were washed with PBS supplemented with 0.05% Tween-20 and exposed to the HRPO-linked anti-mouse secondary antibody for 45 min at room temperature. Color reaction was carried out by incubation for 4 min with liquid DAB+ substrate and counterstaining by Mayer's hematoxylin solution. Staining patterns were divided into three groups following the report by Erkan *et al*[15]; punctate perinuclear staining, diffuse cytoplasmic staining, and negative/faint staining.

Statistical analysis

All experiments were done in duplicate or triplicate. A two-tailed Student's *t*-test was applied for statistical analysis of comparative data. Overall survival curves were generated according to the Kaplan-Meier method. $P < 0.05$ was considered statistically significant.

RESULTS

Efficacy of cytotoxicity induced in pancreatic cancer cell lines by gemcitabine exposure

The response of eight pancreatic cancer cell lines to gemcitabine treatment was investigated using the MTT

Table 1 Classification of the Cell Lines by IC50

	IC50 (μg/mL)	Cell lines
Sensitive group	< 0.1	PK-9, SUIT2
Intermediate group	0.1-100	CFPAC1, PK-1, PK-8, PK-45
Resistant group	> 100	PK-59, PANC-1

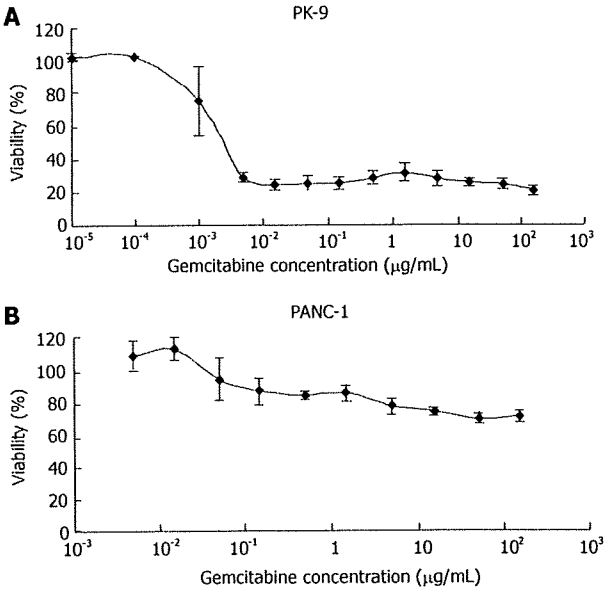

Figure 1 Response to gemcitabine in pancreatic cancer cell lines. Dose-response curves for gemcitabine in PK-9 (A) and PANC-1 (B). These are representative gemcitabine-sensitive and -resistant cell lines, respectively. Each bar represent a standard deviation (SD).

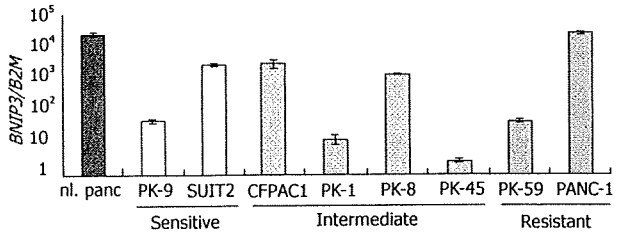

Figure 2 Relative expression levels of *BNIP3* in eight pancreatic cancer cell lines. *BNIP3* expression in pancreatic cancer cell lines as determined by quantitative real-time RT-PCR, and the expression of *B2M* was monitored as the internal control. The cell lines are arranged by IC50 for gemcitabine. No significant correlation was observed between the expression level of *BNIP3* and IC50.

assay. Representative dose-response curves are shown in Figure 1; PANC-1 is a representative resistant cell line, whereas PK-9 is a representative sensitive cell line. The pancreatic cancer cell lines were divided into three groups according to their IC50 values as shown in Table 1. Judging from the IC50 value, two cell lines (PK-9 and SUIT2) showed high sensitivities to gemcitabine; less than 30% of the cells survived in the presence of 0.1 μg/mL of gemcitabine for 72 h (Figure 1A). In contrast, PANC-1 and PK-59 showed very low sensitivity; more than 50% of the cells survived even in the presence of more than 100 μg/mL of the drug for 72 h (Figure 1B). The remaining four cell lines (CFPAC1, PK-1, PK-8 and

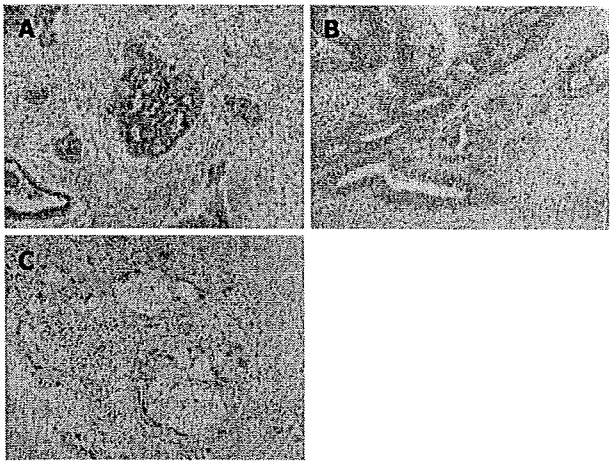

Figure 3 Immunohistochemical analysis of *BNIP3* in primary pancreatic cancer specimens. A monoclonal anti-*BNIP3* antibody (clone Ana 40) was used. A: Punctate perinuclear staining; B: Diffuse cytoplasmic staining; C: Negative/faint staining.

PK-45) showed moderate sensitivity (IC50 values 0.1-100 μg/mL) and were classified as intermediately sensitive cell lines.

BNIP3 expression level is not associated with chemosensitivity of pancreatic cancer to gemcitabine

The expression of *BNIP3* mRNA was examined in all the pancreatic cancer cell lines by quantitative real-time RT-PCR. Among the cell lines analyzed, *BNIP3* expression levels varied by 10^4 fold, as shown in Figure 2. Cell lines were arranged by their IC50 values from left to right. PANC-1 showed the level of *BNIP3* expression comparable to normal pancreas, but PK-45 and PK-1 showed highly suppressed levels of expression. No significant correlation was observed between the expression level of *BNIP3* and the IC50 for gemcitabine.

Next we examined the expression level of *BNIP3* before and after gemcitabine treatment to see if gemcitabine affects the *BNIP3* expression. After administration of 1 μg/mL of gemcitabine to PANC-1, PK-45, PK-9, and SUIT2, the cells were harvested on d 0, 1, 2, and 3. No significant alterations in *BNIP3* expression were observed in these cell lines (data not shown).

Expression level of BNIP3 in pancreatic cancer tissue and correlation with patient survival

To explore the question of whether the expression level of BNIP3 affects the prognosis, we performed immunohistochemical analyses of resected tumor tissues. The staining patterns of BNIP3 were divided into three groups[15]. Representative examples are shown in Figure 3. Among the 22 evaluated specimens, four patients were classified as negative, 11 patients displayed diffuse cytoplasmic staining and 7 showed the punctate perinuclear pattern. The groups were comparable in terms of tumor morphology, stage, and year of treatment including usage of gemcitabine. Although significant differences were not calculated, a tendency between long survival of the patients and negative/faint BNIP3 expression was observed (Figure 4).

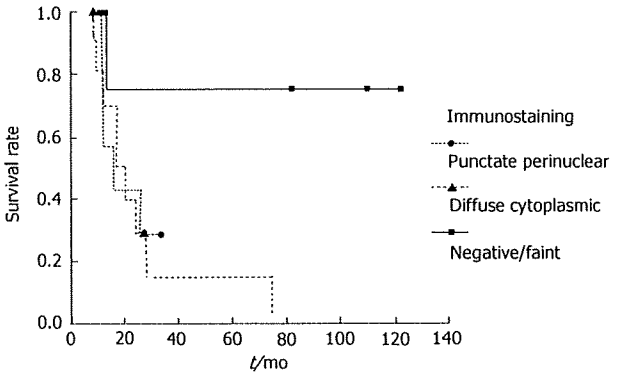

Figure 4 Kaplan-Meyer analysis of the patients with pancreatic cancer by BNIP3 immunostaining patterns. The mean survival time of patients with punctate perinuclear staining group was 20 mo, diffuse cytoplasmic staining group was 26 mo, and negative/faint-staining group was 95 mo. Each group was comparable in terms of tumor stage and adjuvant therapies.

DISCUSSION

Recent studies have reported that *BNIP3* expression is silenced in pancreatic cancer by hypermethylation of its promoter and that loss of *BNIP3* expression contributes to chemoresistance and worsened prognosis[7,8,15]. Furthermore, siRNA-mediated knockdown of BNIP3 caused chemoresistance to gemcitabine[8]. The information was supposed to give us some clues to finding some efficient novel methods for treatment of pancreatic cancer patients. As the first step for invention of such an efficient method for treatment, we tried to confirm these reported results by comparing *BNIP3* expression levels and gemcitabine chemosensitivity in several pancreatic cancer cell lines. However, our results did not support the previous report by Akada *et al*[8]. The expression levels of *BNIP3* in our series of pancreatic cancer cell lines, which were basically the same as used in the previous study, did not reproduce the previous results; the chemosensitivities of pancreatic cancer cell lines to gemcitabine were quite different. Representative gemcitabine sensitive cell lines (CFPAC1 and SUIT2) and the representative resistant cell line (PK-59) were used in both studies, but PK-9, which was previously scored as moderately sensitive to gemcitabine, was actually classified as the most sensitive cell line in our study. PANC-1, which was reported also as "moderate" was the most resistant cell line. In addition, PK-8, which was not tested in the previous study, showed resistance to gemcitabine even with high expression of *BNIP3*. The cause of these discrepancies of the gemcitabine sensitivity between the two studies is unclear; the difference of the methods used in these studies, different culture media, and, probably, cell density, may have caused the discrepancy to some extent. Our immunohistochemical studies also showed results opposite to those reported by Erkan *et al*[15]; loss of BNIP3 expression seemed to correlate with better survival of patients.

In our present study, we could not detect any clear relationship between *BNIP3* expression and chemosensitivity that might associate with a favorable patient survival rate. *BNIP3* is a proapoptotic protein that was considered to have some effect on the chemosensitivity and growth of the pancreatic cancer cell in itself, so it is not difficult to think that overexpression of *BNIP3* leads to cell death and that inhibition of *BNIP3* leads cells survive longer. Our present results by immunostaining supported this idea, albeit no significant difference was obtained. As the number of cases increases, a significant difference will probably be observed. However, pancreatic cancer involves very complicated molecular changes, and those *BNIP3*-positive cancer cells that appear to be weak to the cytotoxic effect and less aggressive may have other genetic and epigenetic changes which can compensate for the proapoptotic effect of *BNIP3* and eliminate the differences between *BNIP3*-positive and -negative cancer cells with regard to chemosensitivity and survival. Furthermore, chemosensitivities can be caused by factors other than those we have discussed so far, such as upregulation of the transportation system to eliminate the drugs from the cells or upregulation of metabolism. Any association between chemosensitivity and BNIP3 expression may yet give us a clue to find a way to invent efficient methods for treatment; further studies are necessary to explain the discrepancy.

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Intraductal papillary-mucinous adenocarcinoma in the remnant pancreas after pancreatoduodenectomy for cancer of Vater's papilla associated with intraductal papillary-mucinous adenoma

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Abstract
A 72-year-old woman, who had undergone pylorus-preserving pancreatoduodenectomy 3 years before for cancer of Vater's papilla associated with a branch-type intraductal papillary-mucinous adenoma (IPMA), developed dilatation of the main duct and a nodular lesion in the remnant pancreas. Total pancreatectomy was performed, which revealed that the lesion was intraductal papillary-mucinous adenocarcinoma (IPMC) with minimal invasion, suggesting the metachronous multicentric occurrence of this intraductal papillary-mucinous neoplasm (IPMN). Because there were no malignant cells at the pancreaticojejunostomy, and because the histological appearance of the main-duct IPMC was different from that of the IPMA in the primary specimen, the main-duct IPMC was thought to be of different origin from the IPMA. These findings suggest that careful surveillance of the gastrointestinal tract and careful follow up are necessary for IPMN, because an IPMN could be associated with other gastrointestinal tract malignancies.

Key words Intraductal papillary-mucinous neoplasm · Multicentric occurrence · Recurrence · Cancer of Vater's papilla

Introduction

Cases of intraductal papillary-mucinous neoplasm (IPMN) were first reported in the 1970s and 1980s.[1,2] In the 1990s, the term "IPMN" was coined, and these neoplasms were established as a special entity among pancreatic neoplasms.[3] At a meeting of international experts on pancreatic precursor lesions held at the Johns Hopkins Hospital from August 18 to 19, 2003, a basic definition of IPMN was worked out.[4]

An IPMN is a primary neoplasm arising in the pancreatic duct that shows a much better prognosis compared with invasive ductal carcinoma of the pancreas. As the number of IPMNs has been increasing, characteristic findings such as metachronous or synchronous multicentric occurrence and concomitant occurrence of the neoplasm in the alimentary tract have been reported. Here we present a case of intraductal papillary-mucinous carcinoma (IPMC) that arose in the remnant pancreas after resection for intraductal papillary-mucinous adenoma (IPMA) associated with cancer of Vater's papilla.

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Case report

A 69-year-old woman suffering from high fever was diagnosed as having obstructive jaundice with acute cholangitis. Then she was referred to our hospital for surgical treatment of the disease. Abdominal computed tomography (CT) and magnetic resonance pancreatography (Fig. 1a) showed a multilocular cystic lesion in the pancreatic head and dilatation of the main pancreatic duct. A tumor of Vater's papilla was revealed simultaneously by endoscopic examination (Fig. 1b). The patient was negative for the tumor markers, carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19–9. Pylorus-preserving pancreatoduodenectomy (PPPD) was performed. Histological examination showed the branch type of intraductal papillary-mucinous adenoma (IPMA) in the pancreatic head (Fig. 1c) and well-differentiated adenocarcinoma (H0, Panc0, Du1, P0, N(–), M(–), Stage II) of Vater's papilla (Fig. 1d). No neoplastic change was found at the cut margin of the pancreas. The patient recovered without any serious complications and continued to undergo routine examination at the outpatient clinic.

Three years later, follow-up abdominal CT (Fig. 2a) showed a 10-mm-wide low-density area in the body of the remnant pancreas and dilatation of the main pancreatic duct (>10 mm), and the CEA and CA19-9

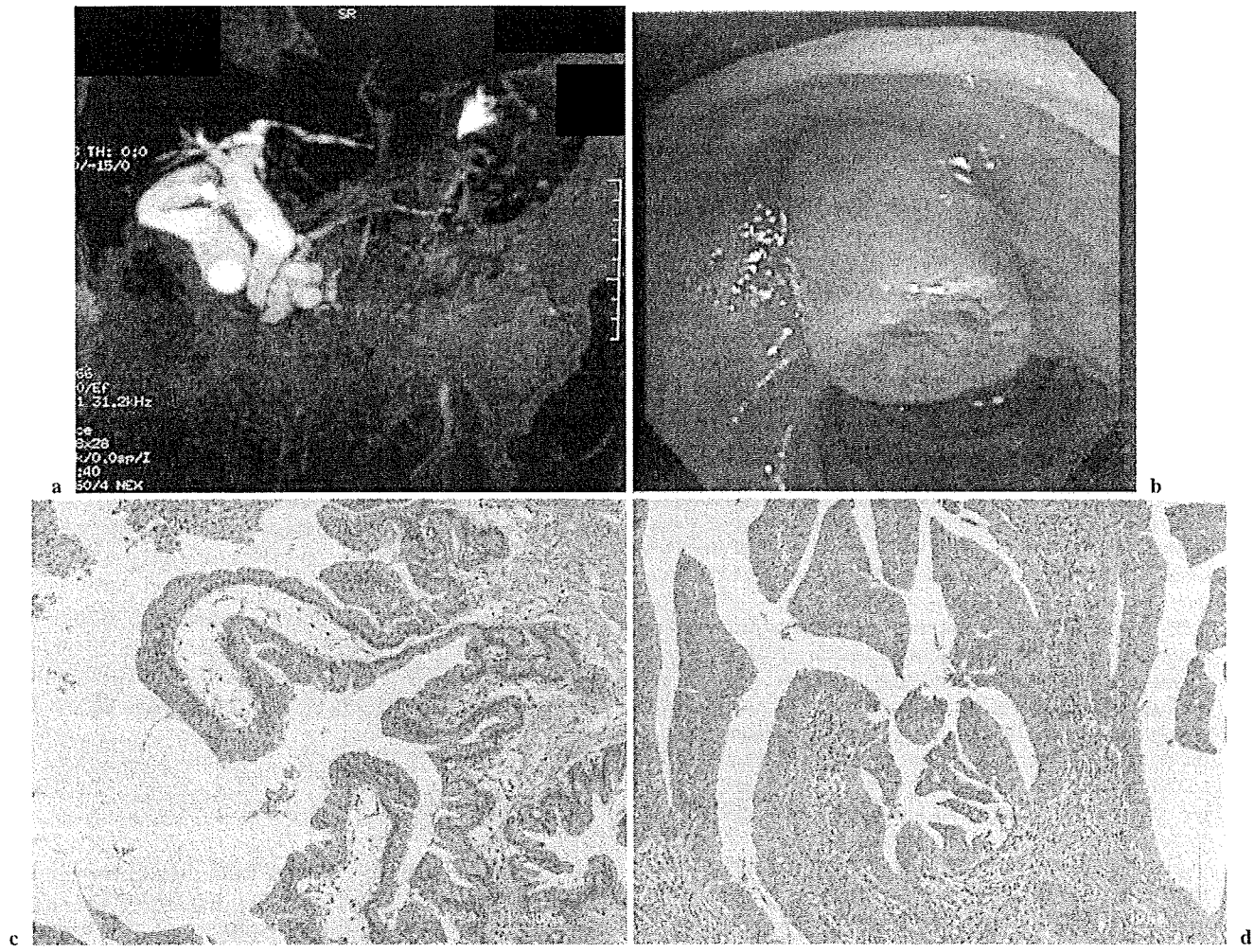


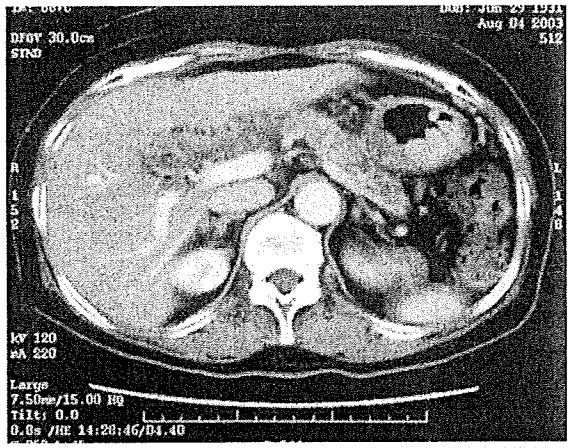
Fig. 1. a Multilocular cystic lesion of the pancreatic head and dilatation of the main pancreatic duct was observed by magnetic resonance pancreatography, and concomitant occurrence of cancer of Vater's papilla was detected by gastrointestinal endoscopy (**b**). Histological examination (H&E, ×100) revealed intraductal papillary-mucinous adenoma (**c**) and cancer of Vater's papilla (**d**)

values had gradually become elevated, to 8.6 ng/ml and 54.7 IU/ml, respectively. Because the recurrence of IPMN or the development of invasive cancer was suspected, total pancreatectomy of the remnant pancreas was performed. Histopathological examination revealed IPMC with minimal invasion[5] (Fig. 2b). No cancer cells were found at the pancreaticojejunostomy, suggesting that the IPMC was not derived from the IPMA resected 3 years before, but that it represented a metachronous multicentric occurrence (pathological mapping is shown in Fig. 2c). Immunohistochemistry was done on the first IPMA (MUC1core, –; MUC2, –; MUC5AC, +) and the second IPMC (MUC1core, +; MUC2, –; MUC5AC, +). The first IPMA was classified as gastric-type, and the second IPMC was not classified to any subtype (uncertain type).[6] Immunohistochemistry for tumor suppressor genes such as *TP16*, *TP53*, *SMAD4*, and *DUSP6* was performed, but no abnormality was observed.

Discussion

IPMN is characterized by the dilatation of the main or branch pancreatic ducts that contain a thick mucoid secretion and is classified as main-duct IPMN or branch-duct IPMN, based on imaging studies or histology.[7] This disease shows various types of histological changes, such as hyperplasia, adenoma, adenocarcinoma, and invasive cancer.

IPMN has a higher resectability rate and more favorable prognosis after resection than invasive pancreatic carcinoma. Recently, it has been recognized that



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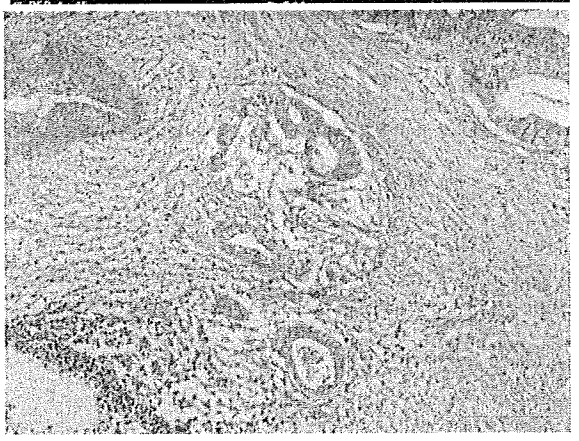

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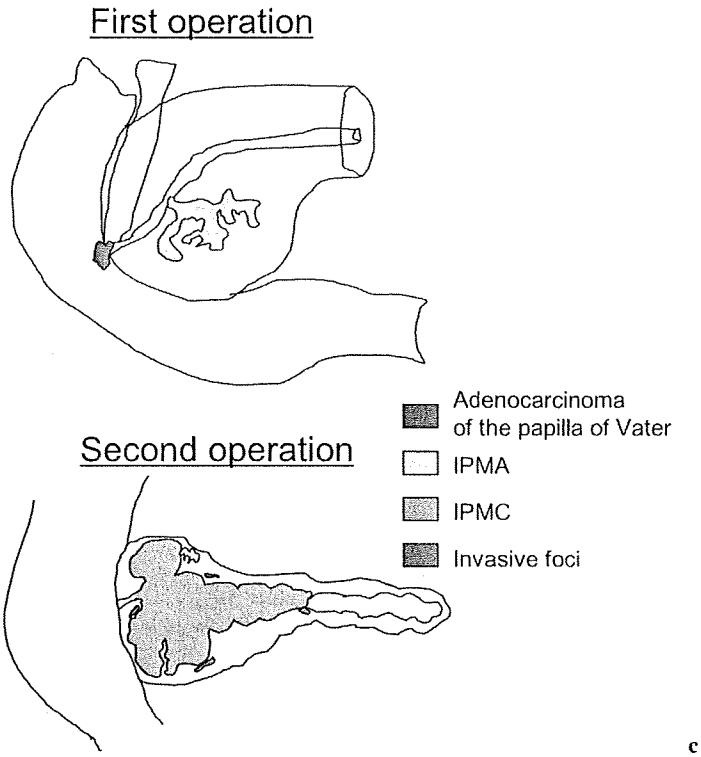

c

Fig. 2. **a** Follow-up computed tomography (CT) showed a low-density area in the body of the remnant pancreas and dilatation of the main pancreatic duct (>10 mm). **b** Histological examination (H&E, ×100) showed intraductal papillary-mucinous carcinoma (*IPMC*) with minimal invasion. **c** Pathological mapping of the cancer of the papilla of Vater, the intraductal papillary-mucinous adenocarcinoma (*IPMA*) at the first operation, and the pancreatic cancer, derived from intraductal papillary-mucinous neoplasm (IPMN,) at the second operation

patients with IPMN frequently have synchronous or metachronous malignancy in various organs, especially in the gastrointestinal tract. Yamaguchi et al.[8] reported that 10% of patients with IPMN had synchronous and metachronous occurrence of invasive cancer of the pancreas at a different site from the IPMN. In addition to its association with the high prevalence of pancreatic cancer, IPMN is reported to be associated with malignant neoplasms in other parts of the gastrointestinal tract.[9,10] On the other hand, cancer of the papilla of Vater is known to be associated only with familial adenomatous polyopsis.[11]

IPMN itself was reported to have synchronous and metachronous multicentric occurrence and to show recurrence after resection.[12,13] Relapse of IPMN is thought to arise from the remnant of the neoplasm at the cut margin, from dissemination in the pancreatic duct, or from multicentric precancerous lesions.[6] In the patient reported here, PPPD was carried out because of cancer of Vater's papilla,[14] but not for the IPMN of the pancreatic head because the primary-branch duct IPMN did not match the indications for surgical resection (i.e., the patient is symptomatic, or the lesions are >3 cm and/or have mural nodules).[7,15] In fact, the histological diagnosis of IPMN at the first surgery was adenoma.

The histological appearance of the IPMN in the main duct 3 years after the PPPD was IPMC with minimal invasion (Fig. 2), different from the first lesion, which was IPMA (Fig. 1). This IPMC development in the remnant pancreas was speculated to be independent from the former IPMA because the histological character and immunohistochemistry profile were different and no neoplastic cells were observed at the cut end of the pancreas. Therefore, this IPMC was thought to have arisen neither from anastomotic recurrence nor from the dissemination of tumor cells in the pancreatic duct, but from multicentric development of the neoplasm.

The number of reports related to the coexistence of multiple IPMN and cancer of the pancreas and other parts of the alimentary tract is increasing. A recent study has suggested that IPMN might form part of the spectrum of lesions encountered in attenuated familial adenomatous polyposis.[16] A common underlying genetic etiology may be present in IPMN and other associated malignancies. Further investigations focusing on this issue are necessary. Because IPMN is a slow-growing tumor with a relatively favorable prognosis, associated malignancies may have potential prognostic significance.

In conclusion, in patients with IPMN, systemic surveillance at the diagnosis of IPMN and careful follow-up are necessary for the early detection of associated malignancies, including lesions in the remnant pancreas.

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Characteristic Clinicopathological Features of the Types of Intraductal Papillary-Mucinous Neoplasms of the Pancreas

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Objectives: Intraductal papillary-mucinous neoplasm (IPMN) of the pancreas encompasses a spectrum of neoplasms with both morphological and immunohistochemical variations of mucin glycoproteins. Recently, a consensus nomenclature and criteria were histologically defined for classifying these variants of IPMNs into gastric, intestinal, pancreatobiliary, and oncocytic types. The purpose of this study was to determine associations between the histological types and clinicopathological features in patients with IPMN.

Methods: Sixty-one patients with IPMN operated upon at Tohoku University Hospital between 1988 and 2006 were retrospectively analyzed.

Results: Our series included 27 gastric-, 29 intestinal-, 4 pancreatobiliary-, and 1 oncocytic-type IPMNs. Statistically, the types of IPMN were significantly associated with the histological diagnoses, macroscopic types, and survival of the patients. Characteristically, the gastric-type IPMNs were likely to be diagnosed as benign, to be confined to branch ducts, and to have fair prognoses. On the other hand, the intestinal-type IPMNs were likely to be diagnosed as malignant, occupy the main duct, and have poor prognoses. Because of the small number of pancreatobiliary-type IPMNs and only 1 case of oncocytic-type IPMN, we were unable to determine any of their clinicopathological characteristics in our series.

Conclusions: Evaluation of the histological types of IPMN may help to predict the clinical course of patients with IPMN and to design improved clinical management for these patients.

Key Words: IPMN, pancreatic cancer, MUC, prognosis, histological type, clinicopathological feature

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Intraductal papillary-mucinous neoplasm of the pancreas (IPMN) is characterized by neoplastic cells of the duct-epithelial lineage with abundant mucin lining the surface of ectatic ducts.[1,2] The neoplastic cells show a variety of atypia ranging from low to high grade, which leads to a diagnosis of adenoma or carcinoma depending on the atypical degree. Sometimes they accompany invasive ductal adenocarcinoma with tubular or mucinous colloid features, which suggest that IPMN corresponds to a precursor lesion and progresses to invasive ductal adenocarcinoma. Since the first definite report of IPMN,[3] IPMNs have shown several histological variations. Recently, a new classification of these histological variations was established based on the morphology of the papillae and their immunohistochemical features of mucin glycoproteins (MUCs).[4] Accordingly, IPMNs are classified into 4 distinct types, which are gastric, intestinal, pancreatobiliary, and oncocytic types. The gastric-type IPMN consists of cells resembling gastric foveolae. They express MUC5AC but are negative for MUC1 and MUC2. The intestinal-type IPMN resembles intestinal villous neoplasms with tall columnar epithelial cells. The neoplastic cells consistently express MUC2 and MUC5AC but are negative for MUC1. The pancreatobiliary-type IPMN consists of cells resembling cholangiopapillary neoplasms and shows complex, thin, and branching papillae. The lesion is at least focally positive for MUC1 and consistently expresses MUC5AC but not MUC2. The oncocytic-type IPMN consists of cells with abundant and intensely eosinophilic cytoplasm and shows complex thick papillae with intra-epithelial lumina, which expresses MUC5AC consistently and MUC1 and MUC2 focally.

Although these histological features of the types of IPMNs seem to be characteristic, their clinicopathological features are not well known.

The purpose of this study was to determine the association between the types and clinicopathological features of IPMNs.

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MATERIALS AND METHODS

Patients

Sixty-one patients with IPMN who underwent surgery at the Department of Gastroenterological Surgery, Tohoku University Hospital, Sendai, Japan, from 1988 to 2006 were retrospectively analyzed. All patients agreed by written consent to the use of their anonymous records and tissues for scientific studies including this study. This study was approved by the Ethics Committee of Tohoku University School of Medicine. Surgical specimens from the patients were fixed in 10% buffered formalin solution and embedded in paraffin for histological analysis.

Immunohistochemistry

Immunohistochemical staining of MUC1-CORE, MUC2, and MUC5AC was performed as described previously.[4]

Statistical Analysis

Statistical analysis was performed on Statview 5.0 software (SAS Institute Inc, Cary, NC). Any probability values <0.05 were considered to be statistically significant.

RESULTS

Clinicopathological Features

Sixty-one patients with IPMNs operated upon consecutively at Tohoku University Hospital between 1988 and 2006 (18 years) were retrospectively analyzed. The patients included 45 men and 16 women with a mean age of 65.3 years (range, 48–79 years). Six patients underwent total pancreatectomy, 37 patients pancreatoduodenectomy, 17 patients distal pancreatectomy, and 1 patient segmental pancreatectomy. The macroscopic types of IPMNs were determined by clinical imaging studies and investigation of resected tumors, according to criteria described previously,[5] as follows: main duct type, 24 tumors, with 14 uniformly dilated type and 10 focally dilated type; branch duct type, 37 tumors, with 31 cystic subbranch type and 6 dilated subbranch type. Histopathologic diagnoses of the resected tumors were made one of the authors (T.F.) according to criteria defined by the Japan Pancreas Society.[1] A diagnosis of intraductal papillary-mucinous adenoma (IPMA) was made for 26 tumors, intraductal papillary-mucinous carcinoma (IPMC) for 29 tumors, and invasive adenocarcinoma being derived from IPMN for 6 tumors, with 3 tubular adenocarcinomas and the remaining 3 mucinous colloid carcinomas. The macroscopic types were found to be significantly associated with histological diagnosis: 20 (83.3%) of 24 tumors with the main duct type and 21 (43.2%) of 37 tumors with the branch duct type were diagnosed as malignant ($P = 0.0019$, χ^2 test).

Types of IPMN

The resected tumors were classified into the 4 distinct histological types of IPMN (ie, gastric, intestinal, pancreatobiliary, and oncocytic types) by their morphology and immunohistochemical reactivity to MUC1-CORE, MUC2, and MUC5AC, according to criteria described previously.[4] Of the 61 tumors, 27 (44.3%) were classified into the gastric type, 29 (47.5%) into the intestinal type, 4 (6.6%) into the pancreatobiliary type, and 1 (1.6%) into the oncocytic type (Fig. 1).

Association Between the Types of IPMN and Clinicopathological Features

The types of IPMN were significantly associated with the histological diagnoses ($P < 0.0001$, χ^2 test) (Table 1): 22

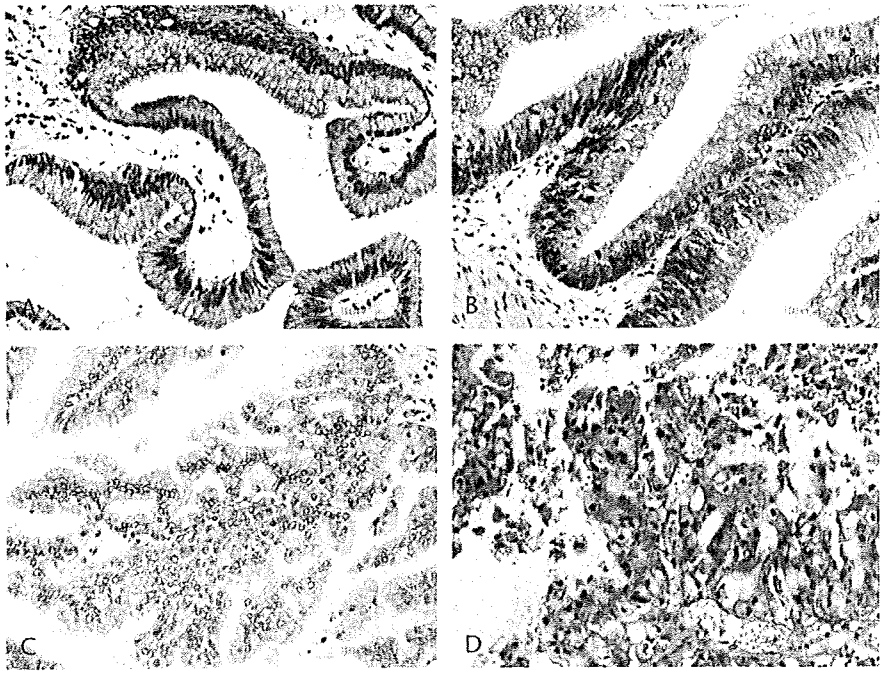

FIGURE 1. Representative histological images of the types of IPMNs: (A) gastric, (B) intestinal, (C) pancreatobiliary, and (D) oncocytic.

TABLE 1. Types of IPMNs and Clinicopathological Features

Type	n	Sex (Male/Female)	Mean Age (yrs)	Mean Survival Period* (mo)	Diagnosis				Macroscopic Type	
					IPMA	IPMC	INV-C	INV-T	Branch	Main
Gastric	27	23:4	66.0	ND†	22	4	0	1	22	5
Intestinal	29	18:11	64.1	52.1	4	21	3	1	11	18
Pancreatobiliary	4	3:1	70.0	ND†	0	3	0	1	3	1
Oncocytic	1	1:0	55.0	ND†	0	1	0	0	1	0
Total	61	45:16	65.3	59.8	26	29	3	3	37	24

*Mean survival period by Kaplan-Meier analysis.
†Not determined because all subjects were censored.
INV-C indicates invasive colloid mucinous adenocarcinoma; INV-T, invasive tubular adenocarcinoma; ND, not determined.

of 27 cases of the gastric type were diagnosed as benign (ie, IPMA), whereas 25 of 29 cases of the intestinal type were diagnosed as malignant, with 21 IPMC, 3 invasive mucinous colloid adenocarcinomas being derived from IPMC, and 1 invasive tubular adenocarcinoma being derived from IPMC. Five gastric-type IPMNs were diagnosed as malignant (4 IPMC and 1 invasive tubular adenocarcinoma being derived from IPMC). The gastric-type IPMCs showed relatively low papillae with severe/high-grade atypia. They diffusely expressed MUC5AC but hardly MUC1 and MUC2 (Fig. 2). The intestinal type IPMAs corresponded to the goblet type, a variation of the intestinal type[4] (Fig. 2).

Four pancreatobiliary-type IPMNs were diagnosed as malignant, including one with invasive tubular adenocarcinoma. One oncocytic type IPMN was diagnosed as IPMC.

Compared with the macroscopic types of IPMN, 22 of the 27 gastric-type IPMNs corresponded to the branch duct type, whereas 18 of the 29 intestinal-type IPMNs to the main duct type, which indicated that the types were closely associated with the macroscopic type ($P = 0.0068$, χ^2 test) (Table 1). Furthermore, the types were associated with the diagnosis in each macroscopic type: 8 of 11 branch duct–type IPMNs of the intestinal type and 4 of 22 of the gastric-type IPMNs were malignant, whereas 1 of 18 main duct–type IPMNs of the intestinal type and 3 of 5 of the gastric-type IPMNs were benign ($P = 0.0020$ for the former and $P = 0.0045$ for the latter, χ^2 test). The oncocytic-type IPMN belonged to the branch duct type. Of 4 pancreatobiliary-type IPMNs, 3 belonged to the branch duct type and 1 to the main duct type.

Invasion was not associated with any specific type of IPMN.

Survival of IPMN

The patients were followed up for their prognosis. The mean follow-up period was 38.1 months (range, 1–176 months). Survival analyses were carried out according to the Kaplan-Meier method. The types of IPMN were associated with survival (Fig. 3A). Patients with intestinal-type IPMNs had a poorer survival than those with gastric-type IPMNs. The 5-year relative survival rates were 62.3% for patients with the intestinal types, whereas 100% for those with the gastric types. The mean survival period for patients with the intestinal-type IPMNs was 52.1 months. A P value could not be calculated because all patients with the gastric-type IPMN were censored. Nine of 29 patients with intestinal-type IPMNs died of the

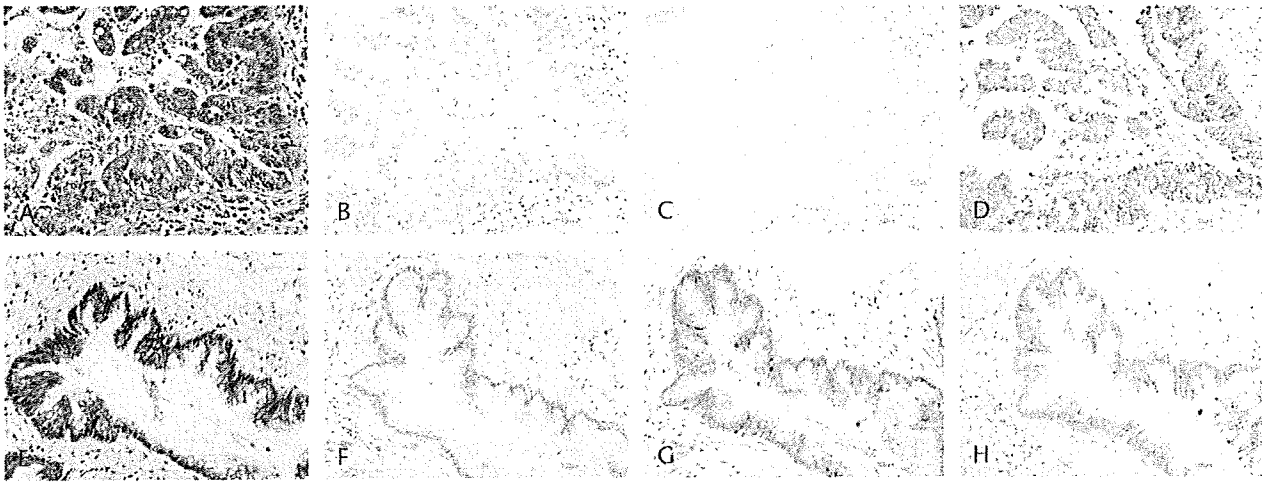

FIGURE 2. Examples of gastric-type IPMC (A–D) and intestinal-type IPMA (E–H). Hematoxylin-eosin staining (A, E) and immunohistochemical staining of MUC1-CORE (B, F), MUC2 (C, G), and MUC5AC (D, H).

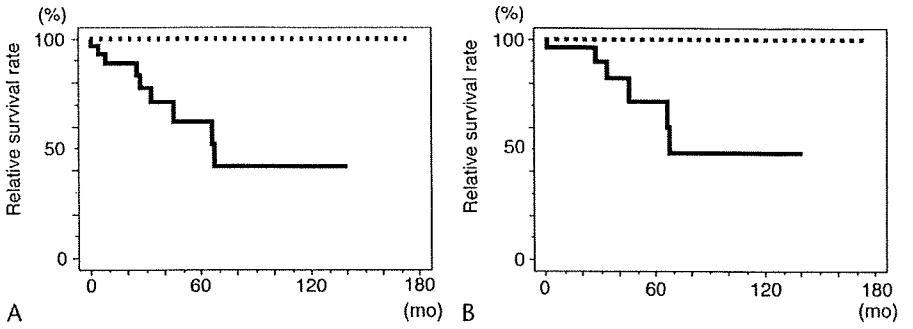

FIGURE 3. Kaplan-Meier survival analysis of patients with gastric- (dashed line) and intestinal-type (solid line) IPMNs: (A) overall survival, (B) survival of patients without invasive carcinoma.

disease during follow-up. All these patients had been diagnosed as having IPMC, including 3 with invasive mucinous colloid carcinomas and 1 with invasive tubular adenocarcinoma. Without the patients with invasive carcinomas, patients with intestinal-type IPMNs still had a poorer survival than those with gastric-type IPMNs (Fig. 3B). Of 4 patients with the pancreatobiliary-type IPMNs, 1 patient died of complications related to the operation and the other 3 patients were alive for 15, 8, and 3 months, respectively, until each observation period was censored. The patient with the oncocytic-type IPMN was alive for 18 months until the observation period was censored.

Diagnosis and the macroscopic types were also associated with survival. According to the diagnosis, the 5-year relative survival rates were 100% for patients with IPMA, 51.9% for patients with IPMC, and 0% for those with invasive adenocarcinoma. A P value could not be calculated because all patients with IPMA were censored. The mean survival period was 55.5 months for patients with IPMC, 20.3 months for patients with invasive mucinous colloid carcinoma, and 5.0 months for patients with invasive tubular adenocarcinoma. The patients with the main duct type revealed significantly poorer survival than those with the branch duct type ($P = 0.0376$ by log-rank test). The mean survival periods were 52.2 months for the main duct type and 64.2 months for the branch duct type. Even in each of the macroscopic type, the histological type seemed to be associated with survival: 3 of 11 patients with the branch duct–type IPMN of the intestinal type died, whereas all 5 patients with the main duct–type IPMN of the gastric type survived.

Sex, age, and operative procedures were not associated with survival.

DISCUSSION

The present retrospective study indicated that the histological types of IPMN are associated with histological diagnoses, macroscopic types, and survival of patients with IPMN. Characteristically, the gastric-type IPMN was likely to be diagnosed as benign, to reveal a fair prognosis, and to be confined to a branch duct. On the other hand, the intestinal-type IPMN was likely to be diagnosed as malignant, to reveal a poor prognosis, and to occupy the main duct. Because the pancreatobiliary and oncocytic types were rare in our series, only 4 cases and 1 case, respectively, we could not estimate any of their clinicopathological characteristics.

Prognoses of the gastric-type IPMNs were fairly good; no patient died of the disease, which indicates that the gastric-type IPMN is less aggressive clinically. Consistently, 22 (81.5%) of the 27 gastric type cases in our series were diagnosed as benign. The remaining 5 cases were diagnosed as malignant, with 4 IPMC and 1 IPMC with invasive tubular adenocarcinoma. These patients were followed up for less than 18 months until being censored in this study; therefore, the long-term prognoses of them have not yet been determined.

Patients with intestinal-type IPMNs had poor prognoses. Consistently, the tumors were associated with malignant diagnosis (ie, IPMC or IPMC with invasion). The intestinal IPMCs accompanied invasive adenocarcinoma more frequently than IPMNs of other types, which certainly worsened the prognosis. Some of the intestinal-type IPMCs without invasion also led to death of the patients, which indicates that the intestinal-type IPMC even without invasion is clinically aggressive and may require careful follow-up or an additional intervention rather than operation only. Adsay et al[6] indicated that the intestinal-type IPMN was associated with mucinous colloid carcinoma that revealed a better prognosis than IPMNs with invasive ductal adenocarcinoma. Although 3 of 4 invasive intestinal-type IPMCs in our series accompanied mucinous colloid carcinoma in their invasive component, we could not statistically evaluate the trend because of the small number of invasive cases.

The gastric-type IPMNs were associated with the branch duct–type IPMN, whereas the intestinal-type IPMNs were associated with the main duct type. Diagnoses were also associated with the macroscopic types. These results indicate that the histological type, macroscopic type, and diagnosis are strongly associated with each other. Patients with the branch duct–type IPMN are known to have a better prognosis than those with the main duct–type IPMNs, which was consistent with the current analysis.[7] Even more notably, the branch duct–type IPMN of the intestinal type was more malignant than that of the gastric type. The main duct–type IPMN of the gastric type was more benign than that of the intestinal type. Our results indicate that we can estimate that a branch duct–type IPMN is likely to be a gastric-type IPMA, whereas a main duct–type IPMN is likely to be an intestinal-type IPMC.

In conclusion, evaluation of the histological types of IPMN may help to predict the clinical course of patients with IPMN and to design better clinical management of these patients.

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