

# Acinar Cell Carcinoma of the Pancreas

## Clinical Analysis of 115 Patients From Pancreatic Cancer Registry of Japan Pancreas Society

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**Objectives:** Acinar cell carcinoma (ACC) of the pancreas is a rare tumor, and many aspects remain unclear because no large-scale clinical studies have been conducted.

**Methods:** The present study investigated the clinical characteristics, treatment, and therapeutic outcomes of 115 patients registered in the Pancreatic Cancer Registry of the Japan Pancreas Society, and therapeutic plans were reviewed.

**Results:** Although ACC has been associated with advanced stage and poor prognosis, this tumor was resectable in 76.5% of the patients, and the 5-year survival rate after resection was favorable, being 43.9%.

**Conclusions:** Confirming the diagnosis of ACC preoperatively is difficult, but this diagnosis should be kept in mind while planning surgery for ordinary pancreatic cancer. Once the diagnosis has been confirmed, a possibility of surgical resection should be pursued to achieve better prognosis. If ACC is unresectable or recurrent, chemotherapy is likely to prove useful. Multidisciplinary therapy centering on the role of surgery will need to be established.

**Key Words:** acinar cell carcinoma

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Acinar cell carcinoma (ACC) is a relatively rare tumor of the pancreas compared with ductal cell carcinoma (DCC) and arises from acinar cells. Acinar cell carcinoma is a well-known entity, owing to its rarity, but for the same reason, a single institution is highly unlikely to treat many patients with ACC, and many issues concerning this tumor remain unclear. In particular, no consensus has been reached on surgical treatment or therapeutic outcomes.

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The present study analyzed 115 patients with ACC accumulated in the Pancreatic Cancer Registry of the Japan Pancreas Society (JPS) for clinical characteristics, treatment, and therapeutic outcomes with reference to the literature.

### MATERIALS AND METHODS

During a period of 23 years from 1981 through 2004, a total of 115 patients with ACC or adenoma were registered in the Pancreatic Cancer Registry of the JPS. On average, approximately 7 to 8 patients were registered annually (Fig. 1). From 1981 to 1999, both adenoma and adenocarcinoma were registered as adenocarcinoma, and so far, only one 60-year-old man in 2003 has been diagnosed with pancreatic acinar cell adenoma. The 115 patients (77 men and 38 women) displayed a mean age of 59.6 years (range, 30–85 years). Of the 115 patients, 112 patients were observed. Of the 112 patients, tumor was resected in 87 patients, and tumor stage was recorded for 74 patients. Among these patients, tumor stage, tumor markers, treatment, and therapeutic outcomes were investigated. Tumor staging including T, N, and M categories was based on the JPS staging system.<sup>1</sup> Survival was calculated using the Kaplan-Meier method, and a log-rank test was used to compare survival based on resection and stage.

### RESULTS

#### Tumor Stage

The breakdown of tumor stages was as follows: stage I, n = 2; stage II, n = 15; stage III, n = 12; stage IVa, n = 21; stage IVb, n = 39; and unknown, n = 26 (Table 1). The annual number of the registration in relation to stage did not have the unevenness. According to JPS T classification system, T4 accounted for 55.3% of the total, and positive portal vein invasion (PV), arterial invasion (A), and extrapancreatic nerve plexus invasion (PL) accounted for 35.1%, 21.1%, and 15.6%, respectively. In terms of N classification, N0 accounted for 57.6% of the total (49/85). In terms of M classification, M0 accounted for 71.3% of the total (77/108). Histologically confirmed infiltration into the main pancreatic duct was seen in 19 (29.2%) of 65 patients.

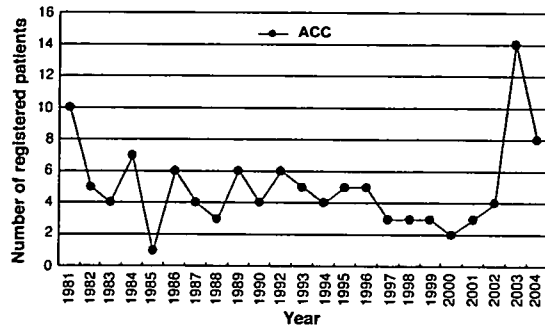


FIGURE 1. Annual shifts in registration count of patients of ACC.

**Tumor Markers**

Figure 2 shows the results of CEA, CA19-9, and SPAN1 analyses for cases registered from 2001 to 2004. Mean values for the 3 markers were 7.6 ng/mL, 26.1 U/mL, and 34.8 U/mL, respectively. Although only 24 patients were registered, no significant correlation was noted between cancer stages and tumor markers, and only 1 case for each marker displayed extremely high levels.

**Therapy Summary**

In the present study, resection was performed on 88 patients (resection rate, 76.5%), palliative operation on 12 patients, and exploratory laparotomy on 4 patients. Details of resections were as follows: total pancreatectomy, 12.3%; pancreatoduodenectomy, 38.6%; distal pancreatectomy, 47.7%; and partial resection of the pancreas, 1.1% (Table 2).

**Therapeutic Outcomes**

Figure 3 shows the survival rate for resected and unresected cases. The 5-year survival rate for the 87 resected cases was 43.9%, with a median survival time (MST) of 41 months. The 5-year survival rate for unresected cases was 0%, with an MST of 3 months. A significant difference was identified between resected and unresected cases ( $P < 0.0001$ ).

Figure 4 shows the survival rate for the 74 resected cases in relation to stage. The 5-year survival rate was 76.9% for stage II, 30.9% for stage III, with an MST of 18 months, 43.9% for stage IVa, with an MST of 41 months, and 25.1% for stage IVb, with an MST of 12 months. A significant difference in survival rate was identified between stages II and III.

Of the registered patients, information about recurrence was obtained from 11 patients. Sites of recurrence was liver ( $n = 8$ ), peritoneum ( $n = 2$ ), or local ( $n = 1$ ). No metastases to lung, brain, or bone were identified.

**DISCUSSION**

A 20-year review by the JPS Pancreatic Cancer Registry<sup>2,3</sup> included only 87 cases of ACC, and ACC accounted for 1 in every 119 cases of DCC, compared with

10,336 cases of DCC. In the literature, ACC accounts for about 1% to 2% of all pancreatic cancers,<sup>4-6</sup> and only small-scale studies have examined ACC, such as Sumii et al<sup>6</sup> ( $n = 6$ ), Klimstra et al<sup>7</sup> ( $n = 28$ ), and Webb<sup>8</sup> ( $n = 11$ ).

In the present study, 67% of the 115 ACC patients were men. The male preponderance was consistent with previous studies conducted by Sumii et al<sup>6</sup> and Klimstra et al<sup>7</sup> (100% and 86%, respectively). Mean age is lower for ACC than for DCC, and patients younger than 30 years old were included in this study, as previously reported. A 9-year-old patient was reported by Osborne et al,<sup>9</sup> and a 25-year-old patient was reported by Webb.<sup>8</sup>

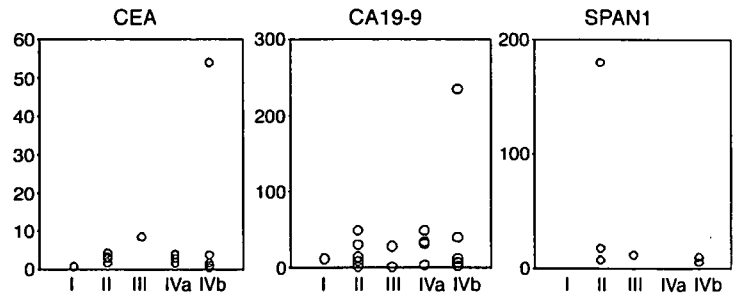
Acinar cell carcinoma has so far been considered a cancer with poor prognosis, because, at the time of diagnosis, the tumor size is often large, vascular invasion is common, and hepatic and lymph node metastases occur early.<sup>5,10</sup> The results of the present review show that T4 accounts for 55.3% of the total, but percentages of positive PV, A, and PL were low at 35.1%, 21.2%, and 15.6%, respectively. With DCC, T4 accounted for 74.8% of the total, and the percentages of positive PV, A, and PL were 59.6%, 39.9%, and 31.9%, respectively,<sup>2,3</sup> indicating that vascular invasion is not necessarily common in ACC.

In regard with lymph node metastasis, N0 accounted for 57.6% of the total for ACC and 32.0% of the total for DCC, and M1 accounted for 28.7% of the total for ACC and 38.2% of the total for DCC. These findings suggest that the incidence of metastasis to lymph node and distant organs for ACC is lower than that for DCC.

TABLE 1. Pancreatic Acinar Cell Carcinoma Stage Among Registered Patients

JPS T classification system	Portal vein invasion		
T1	2 (2.1)	PV (-)	63 (64.9)
T2	23 (24.5)	PV (+)	34 (35.1)
T3	17 (18.1)	PV unknown	18
T4	52 (55.3)	Arterial invasion	
T unknown	21	A (-)	78 (78.8)
JPS N classification system		A (+)	21 (21.2)
N0	49 (57.6)	A unknown	16
N1	9 (10.6)	Extrapancreas nerve plexus invasion	
N2	13 (15.3)	PL (-)	65 (84.4)
N3	14 (16.5)	PL (+)	12 (15.6)
N unknown	30	PL unknown	38
JPS M classification system		Main pancreatic duct progress	
M0	77 (71.3)	MPD (-)	46 (70.8)
M1	31 (28.7)	MPD (+)	18 (27.7)
M unknown	7	MPD (borderline)	1 (1.5)
Stage		MPD unknown	50
I	2 (2.2)		
II	15 (16.9)		
III	12 (13.5)		
IVa	21 (23.6)		
IVb	39 (43.8)		
Stage unknown	26		

Values are given as n (%).



**FIGURE 2.** Relationship between acinar cell carcinoma stages and tumor markers for patients registered from 2001 to 2004.

CEA	n = 22	Mean, 7.6 ng/mL (range, 1 – 57)
CA19-9	n = 24	Mean, 26.1 U/mL (range, 1 – 235)
SPAN1	n = 7	Mean, 34.8 U/mL (range, 6 – 180)

Although invasion into the main pancreatic duct is reportedly lower for ACC than for DCC, which comes from the pancreatic ductal system,<sup>11,12</sup> histologically confirmed invasion into the main pancreatic duct was seen in 29.2% of the registered ACC patients, suggesting that invasion into the main pancreatic duct is not rare in ACC. As far as preoperative pancreatocholangiography findings for ACC are concerned, nonspecific findings such as interruption of the pancreatic and bile ducts due to tumor compression are seen, and no distinct abnormal findings are present in many patients.<sup>12,13</sup> Hence, even when interruption and blocking of pancreatic ducts is confirmed by preoperative pancreatocholangiography, ACC cannot be excluded, making ACC difficult to differentiate from DCC.

As far as tumor markers are concerned, levels of CEA, CA19-9, and SPAN1 are abnormally high in DCC, but low in ACC. However, although we can say that these low levels of tumor makers are characteristic of ACC, such levels are not useful for differential diagnosis. As for other tumor markers,

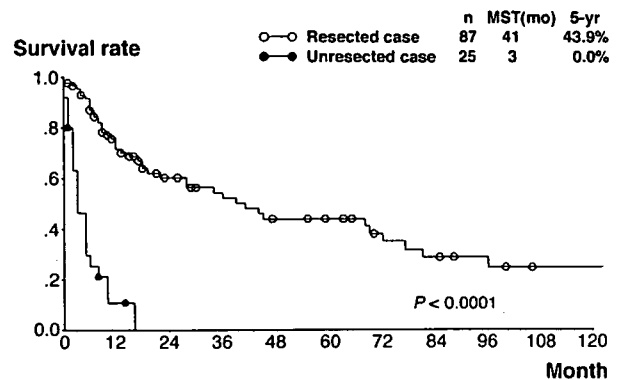
high levels of  $\alpha$ -fetoprotein (AFP) are considered rare in pancreatic cancer,<sup>14–16</sup> but according to a study by Shimizu et al,<sup>17</sup> 11 of 41 pancreatic tumors displaying high levels of AFP were ACC (27%). Because ACC only accounts for a small percentage of overall pancreatic cancers, tumors showing high levels of AFP display increased frequency of ACC. Consequently, high AFP in pancreatic tumor may suggest ACC. Studies have also documented that ACC occasionally produces pancreatic enzymes such as lipase, amylase, and elastase I and is accompanied by systemic adiponecrosis, arthritis, or symptoms associated with functioning tumors.<sup>7,8,18</sup> In the future, investigation of exocrine function and AFP will be necessary.

Previously, ACC has often been accompanied by widespread metastasis at the time of diagnosis, and surgery was not indicated, resulting in ACC being considered a carcinoma with extremely poor prognosis.<sup>4,7,8</sup> However, because of recent advances in diagnostic imaging and surgical techniques, resection has been actively performed, and long-term survival has been reported.<sup>10,19–22</sup> In terms of surgery, the same operations for DCC have been used, such as pancreatoduodenectomy and distal pancreatectomy, and surgical resection is the only option that can lead to long-term survival. The present results show that resection was

**TABLE 2.** Summary of Pancreatic Acinar Cell Carcinoma Therapy and Resection Techniques

Approach to treatment	
Resection	88 (76.5)
Palliative operation	12 (10.4)
Exploratory laparotomy	4 (3.5)
Non operation	10 (6.7)
Non operative autopsy	1 (0.9)
Operative procedure for resection in 88 patients	
Total pancreatectomy	11 (12.3)
Pancreatoduodenectomy	24 (27.3)
Pancreatoduodenectomy (subtotal)	1 (1.1)
Pylorus-preserving pancreatoduodenectomy	8 (9.1)
Substomach-preserving pancreatoduodenectomy	1 (1.1)
Distal pancreatectomy (tail)	5 (5.7)
Distal pancreatectomy (body-tail)	33 (37.5)
Distal pancreatectomy (subtotal)	3 (3.4)
Spleen-preserving distal pancreatectomy	1 (1.1)
Partial resection	1 (1.1)

Values are given as n (%).



**FIGURE 3.** Comparison of survival rate between resected and unresected acinar cell carcinoma.

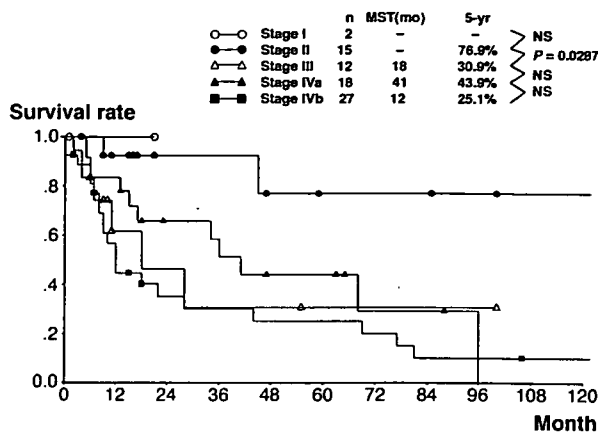


FIGURE 4. Pancreatic acinar cell carcinoma: comparison of survival in relation to tumor stage among resected cases (n = 74).

performed in 76.5% of the total, much higher than in DCC where resection was performed in about 20% to 30% of the total. One reason for the high rate of resection is that vascular invasion and lymph node metastasis are less likely to occur, and invasion to the surrounding tissue is minimal. Among the reported resected cases, carcinoma was generally solitary and large but grew by compressing against the surrounding tissue and was often covered by a fibrous capsule. Acinar cell carcinoma reportedly often arises in the pancreatic head.

Chemotherapy and radiotherapy are other therapeutic options, but insufficient data have been accumulated on the efficacy of these therapies for ACC. Although some studies have reported that chemotherapy was ineffective in the treatment of ACC,<sup>5,7</sup> various chemotherapies have been performed in other studies, such as systemic administration, intra-arterial injection, and intraperitoneal administration, and efficacy and long-term survival have been documented.<sup>10,15,16,22–24</sup> Even when long-term survival cannot be achieved, tumor reduction was achieved and quality of life improved. Unlike DCC, ACC is often a hypervascular tumor, as are metastatic lesions,<sup>24,25</sup> and as a result, antitumor agents readily reach the tumor. Because long-term survivors are all resected cases, surgical resection should be regarded as a treatment of choice. However, when ACC recurs after resection or is unresectable, chemotherapy may be a valid option, although therapeutic regimens and methods vary.

To the best of our knowledge, no reports have described radiotherapy alone, but several studies have documented the efficacy of radiochemotherapy.<sup>22,23</sup> The radiosensitivity of ACC and efficacy of radiotherapy against ACC have not been clarified.

Like DCC, prognosis has been considered poor for ACC. Webb<sup>8</sup> reported an MST of 4.3 months, and Cubilla and Fitzgerald<sup>4</sup> reported a mean survival time of 7 months, but these studies described patients with unresectable ACC. Sumii et al<sup>6</sup> studied 6 ACC patients, including 4 resectable cases, and reported a median survival of 358 days and 1-year survival of 50%. Klimstra et al<sup>7</sup> described 28 ACC patients,

including 18 resectable cases, in which mean survival was 18.1 months and 1-year survival of 56.5%.

In the present study, the 5-year survival rate of 87 resected patients was 43.9%, with MST of 41 months, and these figures are much more favorable when compared with resected DCC patients with a 5-year survival rate of 12.2% and MST of 8.7 months.<sup>2,3</sup> Among resected cases, survival rate for ACC in any stage was higher when compared with DCC, for which survival has been reported as stage I, 58.6%; stage II, 51.0%; stage III, 25.9%; stage IVa, 11.9%; and stage IVb, 2.8%.<sup>2,3</sup> Also, the 5-year survival rate for stage IVb ACC was relatively favorable at 25.1%, with an MST of 12 months. These findings suggest that the survival rate is better for ACC than for DCC in any stage, and even in advanced cases, survival can be improved by resection. Surgery should thus be actively performed in the treatment of ACC. Although ACC has been considered a pancreatic cancer with poor outcomes, surgery appears to be associated with much better prognosis than anticipated.

## CONCLUSIONS

The present study reviewed the clinical characteristics, treatment, and therapeutic outcomes of ACC. Compared with DCC, ACC has been considered a cancer with poor prognosis, but the present study showed that ACC can be resected in many cases and prognosis is greatly improved after resection. Surgery should thus be actively performed in the treatment of ACC. Although ACC is a rare tumor that cannot be easily differentiated from other common pancreatic tumors preoperatively, surgeons should keep ACC in mind. When a pancreatic tumor has proved to be ACC, surgeons should resect it actively using the same procedures as for DCC. Furthermore, even if ACC is unresectable or recurs postoperatively, chemotherapy should be considered. Future studies of more patients and establishment of multidisciplinary therapy centering on the role of surgery are warranted.

## ACKNOWLEDGMENT

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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 rationale 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. © 2007 Excerpta Medica Inc. All rights reserved.

**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 rationale 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 (0.0%)	10 (10.0%)	NS
Adeno	18 (85.7%)	3 (14.3%)	0 (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 (0.0%)	8 (10.0%)	
Anaplastic	0 (0.0%)	2 (10.0%)	0 (0.0%)	2 (10.0%)	
Acinar cell carcinoma	3 (10.0%)	0 (0.0%)	0 (0.0%)	3 (10.0%)	
Undifferentiated	1 (10.0%)	0 (0.0%)	0 (0.0%)	1 (10.0%)	
TS1 ( $\leq$ 2.0 cm)	61 (88.4%)	8 (11.6%)	0 (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 (0.0%)	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 (0.0%)	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 (0.0%)	18 (10.0%)	NS
D1	8 (61.5%)	5 (38.5%)	0 (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(+)	.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.



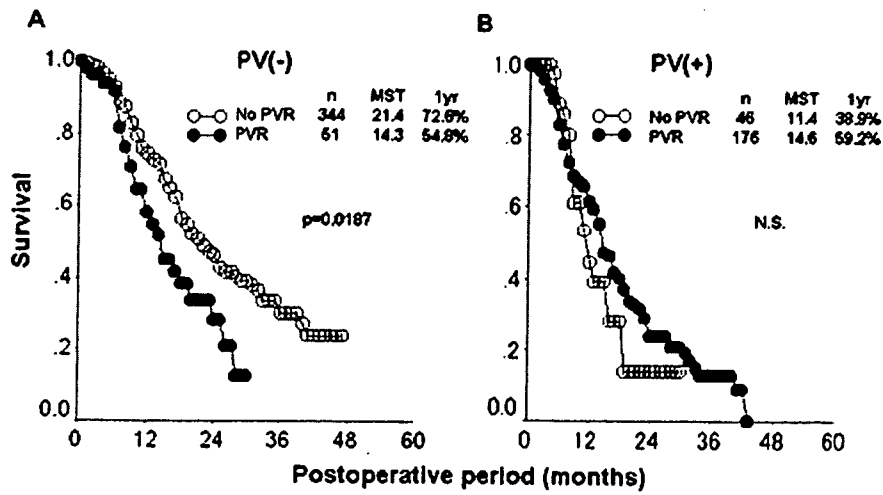


Fig. 1. Portal vein (PV) resection and cumulative survival of patients with International Union Against Cancer stage IIa and IIb. MST = median survival time.

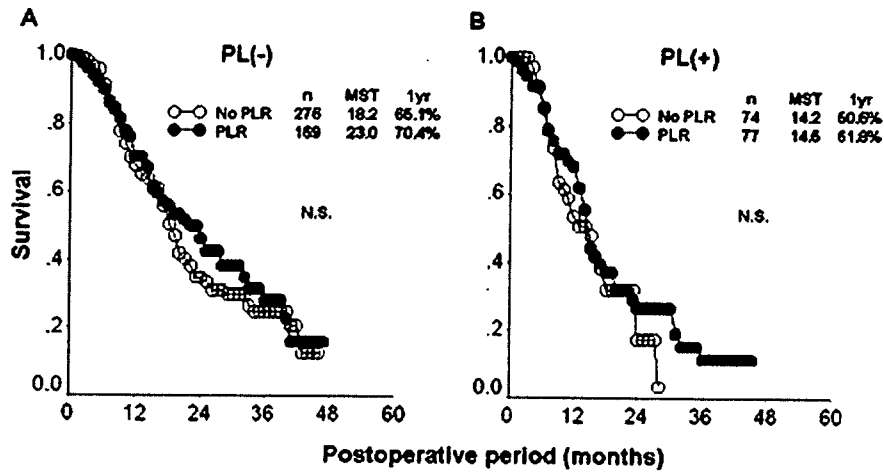


Fig. 2. Plexus resection and cumulative survival of patients with International Union Against Cancer stage IIa and IIb. MST = median survival time; PL = peripancreatic plexus infiltration; PLR = peripancreatic plexus resection.

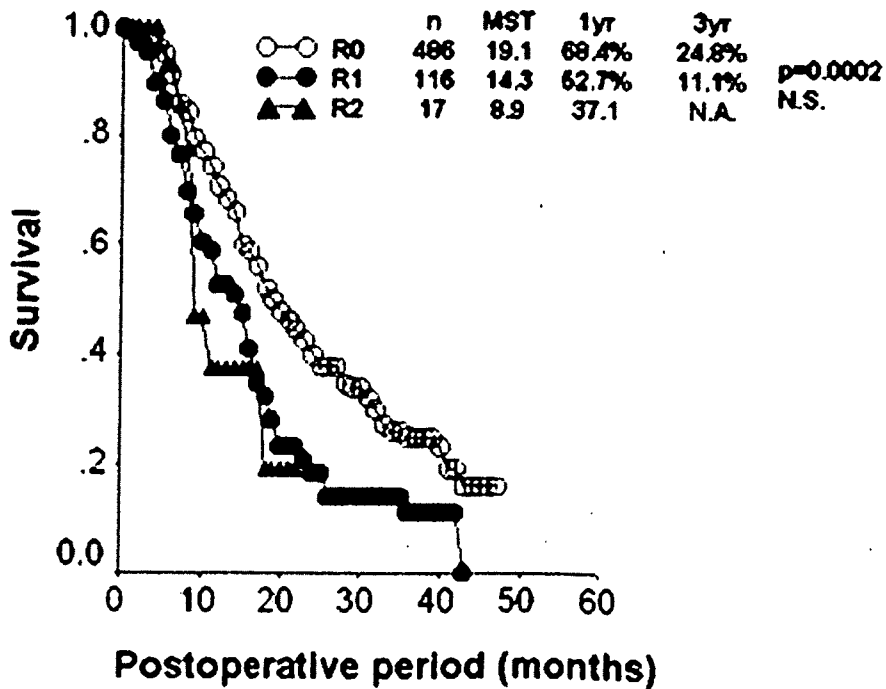


Fig. 3. R level and survival after pancreatotomy for International Union Against Cancer stage IIa and IIb disease. MST = median survival time.

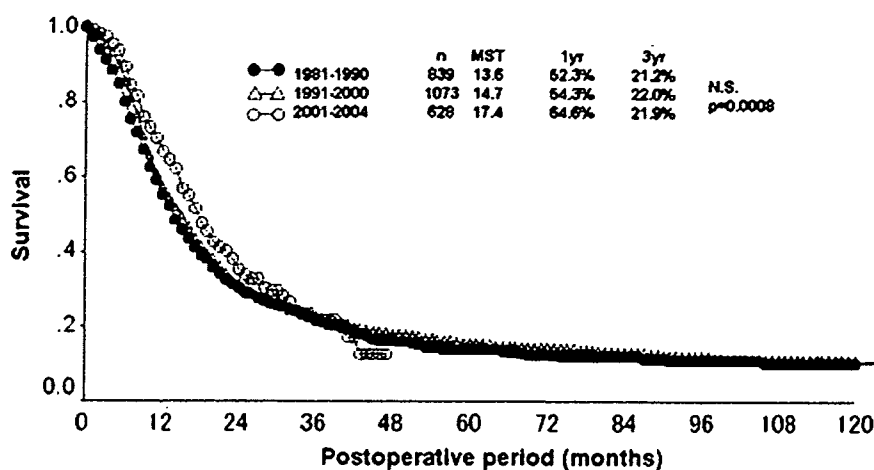


Fig. 4. Trend of survival after pancreatectomy for International Union Against Cancer stage IIa and IIb disease. MST = median survival time.

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

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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.

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

### 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<sup>[1]</sup>. The expression of BNIP3 is increased under hypoxic conditions by a transcription factor, hypoxia-inducible factor 1 $\alpha$  (*HIF1* $\alpha$ )<sup>[2,3]</sup>, 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<sup>[4,5,6]</sup>.

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<sup>[7]</sup>. 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'-difluorodeoxycytidine (Gemzar, Eli-Lilly, Indianapolis, IN), a novel pyrimidine nucleoside analogue<sup>[8]</sup>. It was expected that downregulation of proapoptotic *BNIP3* might contribute to chemoresistance, 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<sup>[9,10]</sup>. 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<sub>2</sub> with humidity at 37°C.

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

To assess cell proliferation, the MTT test was used as described previously<sup>[11]</sup>. Cells were resuspended in fresh medium and seeded in 96-well plates at concentration of  $5 \times 10^3$  cells/well. Cells were incubated for 24 h at 37°C, and then gemcitabine was added to each well at various concentrations. Each plate was incubated at 37°C for 72 h. An aliquot of 10  $\mu$ L 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°C 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  $\mu$ g total RNA and Super Script II Reverse Transcriptase (Invitrogen, Carlsbad, CA) by the methods described previously<sup>[12]</sup>. 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'-agaagccctgttggtatcttggtg-3' (reverse primer), 5'-tctcactgtgacagtcacctcgc-3' (TaqMan probe). These primers were purchased from Nihon Gene Research Laboratories (Sendai, Japan). Expression of the  $\beta$ 2-microglobulin (*B2M*) gene was monitored as an internal control, and the nucleotide sequences for the primers and the probe were described previously<sup>[13]</sup>. Amplifications were carried out in the reaction mixture in 25  $\mu$ L containing 5  $\mu$ L of cDNA samples and 12.5  $\mu$ L of 2  $\times$  Absolute QPCR ROX Mix (ABgene, Epsom, UK), and the final concentration of 0.2  $\mu$ mol/L of each primer pair and 0.4  $\mu$ mol/L of the probe were added in a program comprised of 2 min at 50°C, 15 min at 95°C, followed by 40 cycles consisting of 15 s at 95°C and 1 min at 60°C. 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<sup>[14]</sup>. 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<sub>2</sub>O<sub>2</sub> solution in 30% methanol. After an overnight incubation with mouse anti-BNIP3 antibody clone Ana 40 (Sigma-Aldrich, St. Louis, MO) at 4°C, 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*<sup>[5]</sup>, 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

	IC50 ( $\mu\text{g/mL}$ )	Cell lines
Sensitive group	< 0.1	PK-9, SUI2
Intermediate group	0.1-100	CFPAC1, PK-1, PK-8, PK-45
Resistant group	> 100	PK-59, PANC-1

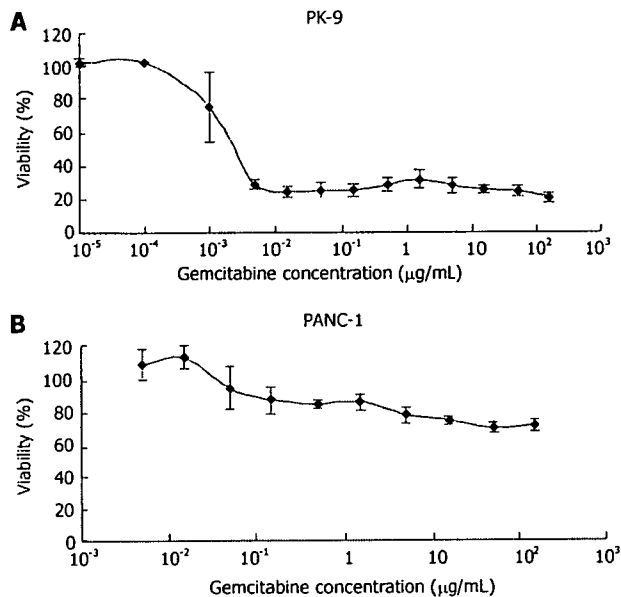


Figure 1 Response to gemcitabine in pancreatic cancer cell lines. Dose-response curves for gemcitabine in PK-9 (A) and PANC-1 (B). These are representative gemcitabine-sensitive and -resistant cell lines, respectively. Each bar represent a standard deviation (SD).

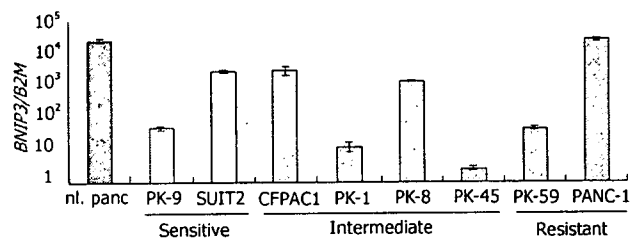


Figure 2 Relative expression levels of *BNIP3* in eight pancreatic cancer cell lines. *BNIP3* expression in pancreatic cancer cell lines as determined by quantitative real-time RT-PCR, and the expression of *B2M* was monitored as the internal control. The cell lines are arranged by IC50 for gemcitabine. 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 SUI2) showed high sensitivities to gemcitabine; less than 30% of the cells survived in the presence of 0.1  $\mu\text{g/mL}$  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  $\mu\text{g/mL}$  of the drug for 72 h (Figure 1B). The remaining four cell lines (CFPAC1, PK-1, PK-8 and

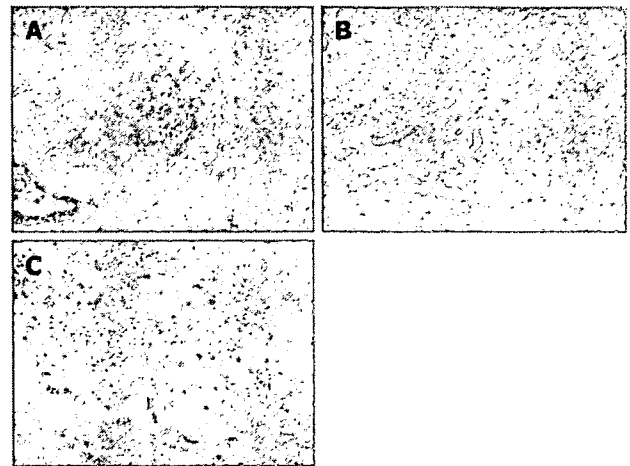


Figure 3 Immunohistochemical analysis of *BNIP3* in primary pancreatic cancer specimens. A monoclonal anti-*BNIP3* antibody (clone Ana 40) was used. A: Punctate perinuclear staining; B: Diffuse cytoplasmic staining; C: Negative/faint staining.

PK-45) showed moderate sensitivity (IC50 values 0.1-100  $\mu\text{g/mL}$ ) 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  $\mu\text{g/mL}$  of gemcitabine to PANC-1, PK-45, PK-9, and SUI2, 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<sup>[15]</sup>. 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).

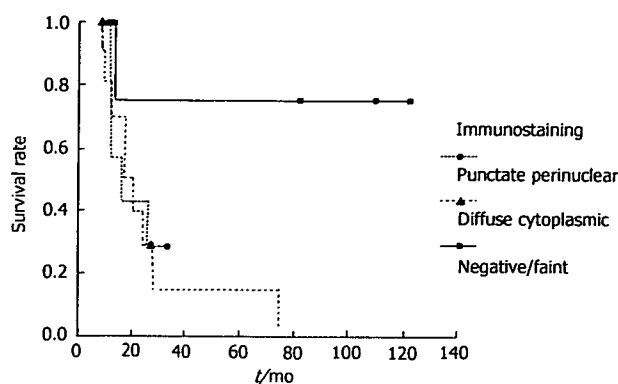


Figure 4 Kaplan-Meier analysis of the patients with pancreatic cancer by BNIP3 immunostaining patterns. The mean survival time of patients with punctate perinuclear staining group was 20 mo, diffuse cytoplasmic staining group was 26 mo, and negative/faint-staining group was 95 mo. Each group was comparable 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<sup>[7,8,15]</sup>. Furthermore, siRNA-mediated knockdown of *BNIP3* caused chemoresistance to gemcitabine<sup>[8]</sup>. 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*<sup>[8]</sup>. 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*<sup>[15]</sup>; 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.<sup>1,2</sup> In the 1990s, the term "IPMN" was coined, and these neoplasms were established as a special entity among pancreatic neoplasms.<sup>3</sup> 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.<sup>4</sup>

An IPMN is a primary neoplasm arising in the pancreatic duct that shows a much better prognosis com-

pared 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.

### Case report

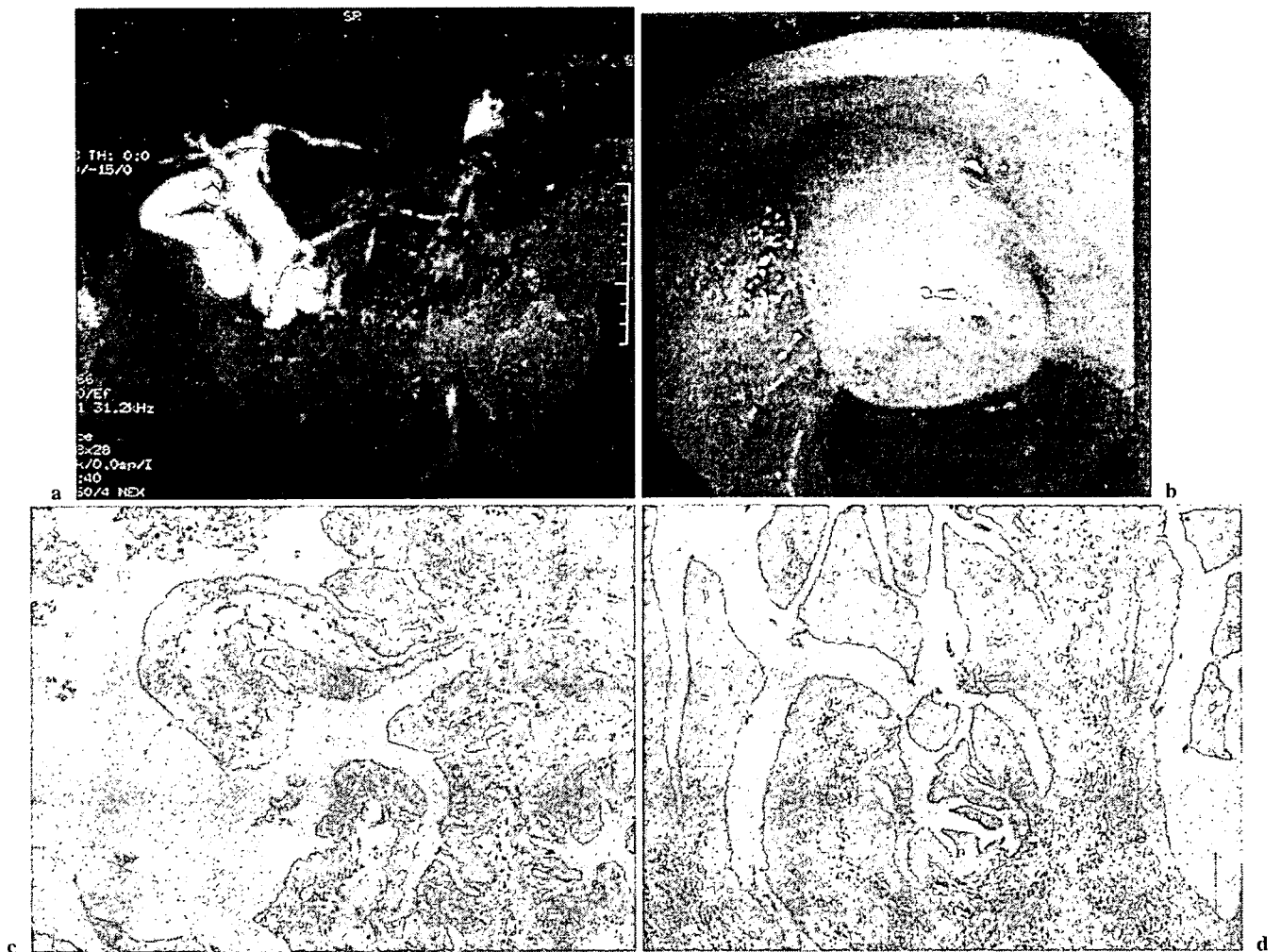
A 69-year-old woman suffering from high fever was diagnosed as having obstructive jaundice with acute cholangitis. Then she was referred to our hospital for surgical treatment of the disease. Abdominal computed tomography (CT) and magnetic resonance pancreatography (Fig. 1a) showed a multilocular cystic 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

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**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 gas-

trointestinal endoscopy (**b**). Histological examination (H&E,  $\times 100$ ) revealed intraductal papillary-mucinous adenoma (**c**) and cancer of Vater's papilla (**d**)

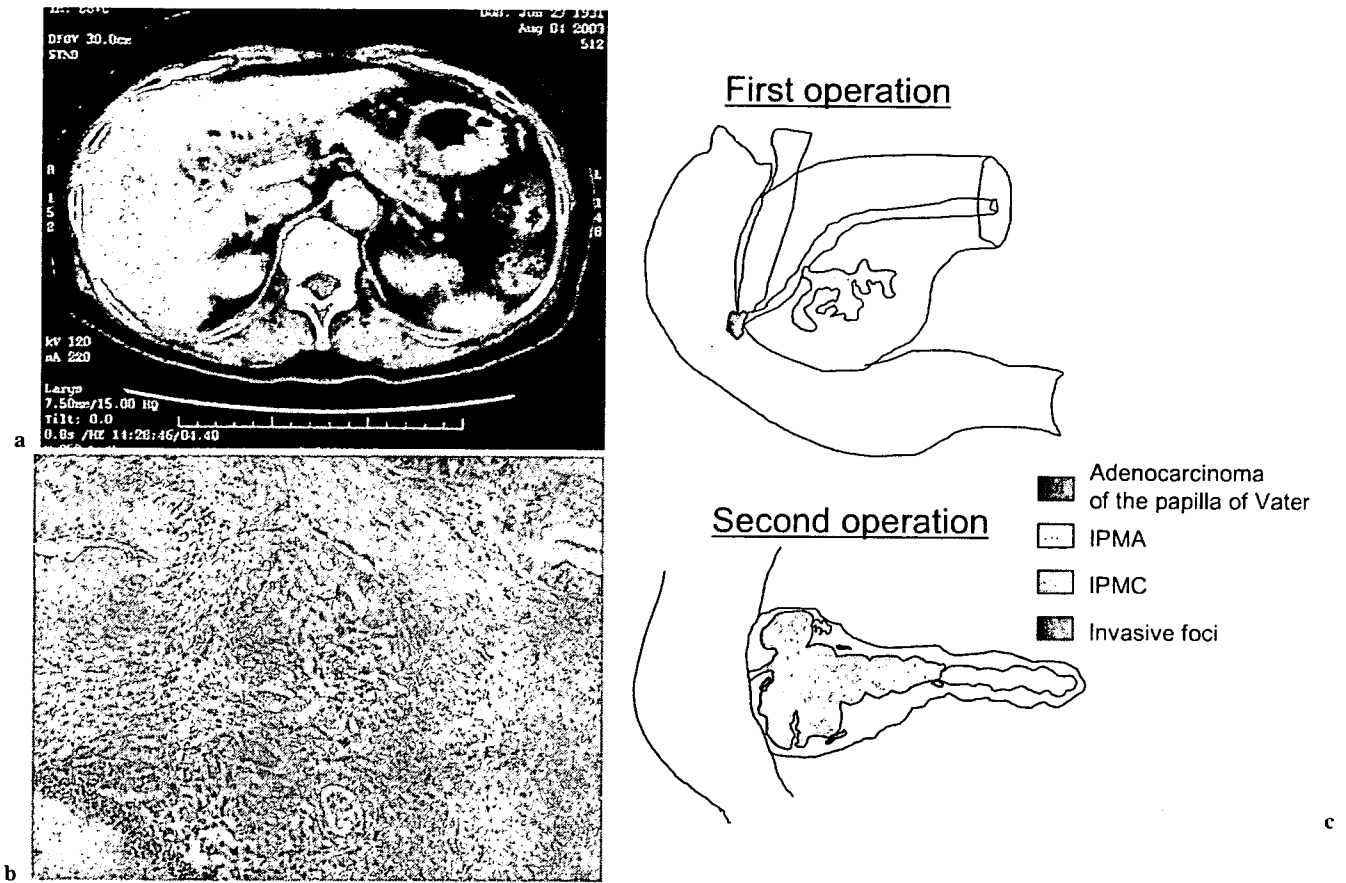
values had gradually become elevated, to 8.6 ng/ml and 54.7 IU/ml, respectively. Because the recurrence of IPMN or the development of invasive cancer was suspected, total pancreatectomy of the remnant pancreas was performed. Histopathological examination revealed IPMC with minimal invasion<sup>5</sup> (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).<sup>6</sup> Immunohistochemistry for tumor suppress-

or 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.<sup>7</sup> 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



**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,  $\times 100$ ) 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.<sup>8</sup> 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.<sup>9,10</sup> On the other hand, cancer of the papilla of Vater is known to be associated only with familial adenomatous polyposis.<sup>11</sup>

IPMN itself was reported to have synchronous and metachronous multicentric occurrence and to show recurrence after resection.<sup>12,13</sup> 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.<sup>6</sup> In the patient reported here, PPPD was carried out because of cancer

of Vater's papilla,<sup>14</sup> 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).<sup>7,15</sup> 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.<sup>16</sup> 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.<sup>1,2</sup> 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,<sup>3</sup> 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).<sup>4</sup> 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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