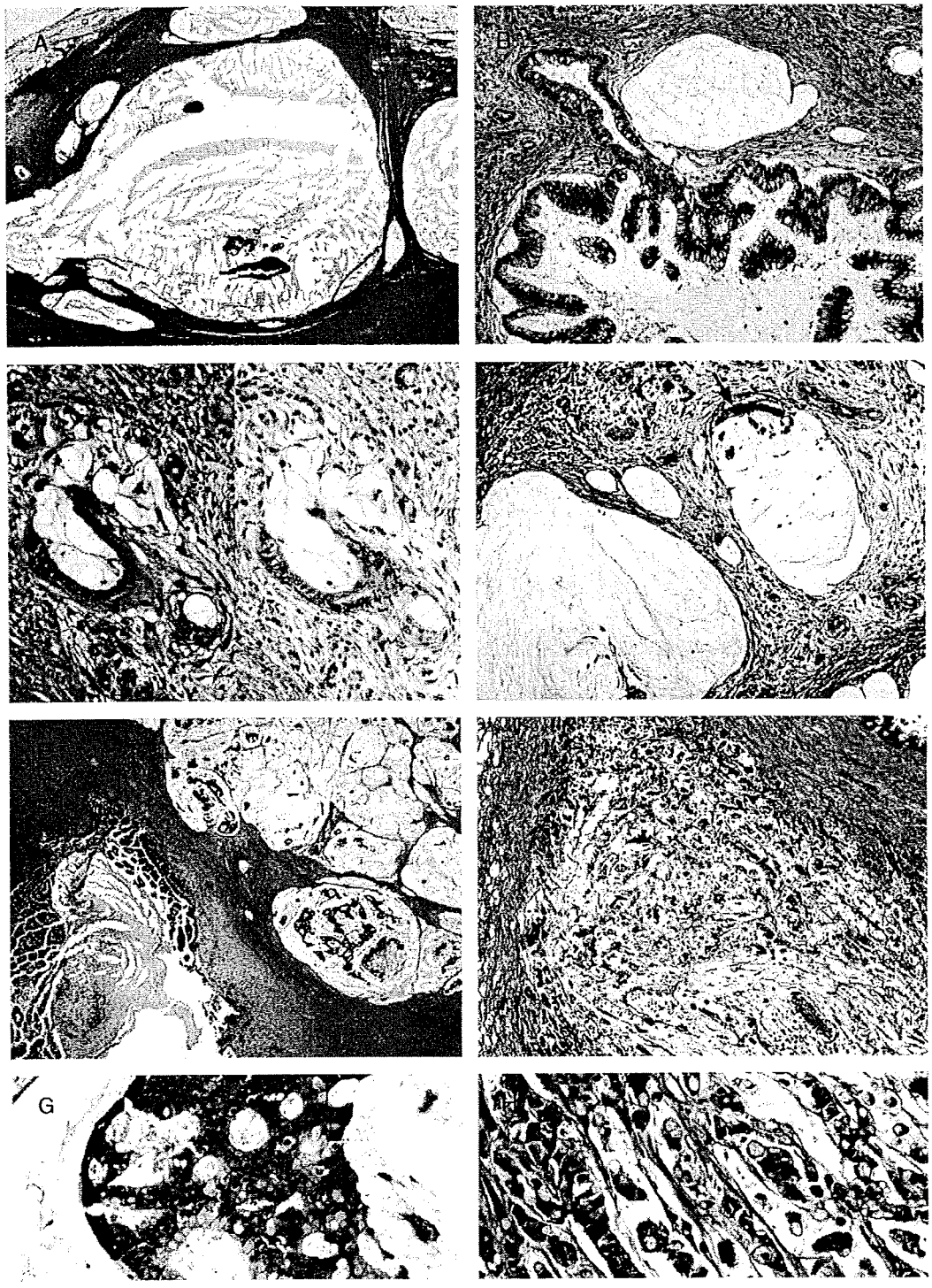

FIGURE 2. Histopathologic features of "mucous rupture" and "infiltrative growth" of mucinous carcinoma in I-IPMC. A to D, "Mucous rupture" pattern. Part of the pancreatic duct is disrupted and mucus leakage is evident. Variable sizes of mucus lakes without viable cancer cells floating are observed (A–C). A small duct covered by elastic fibers (C right column; elastica stain) is broken and the mucus leaks to form mucus lake (C). A small number of cancer cells (arrow) are floating in mucus lakes, which is described as "mucous rupture with cellular component." We could not observe any floating cancer cells in mucus lakes other than this cluster of cancer cells (arrow) in the entire lesion of the I-IPMC (D). E to H, "Infiltrative growth" of mucinous carcinoma. Many cancer cells floating in mucus lakes (E, G) or infiltrating features of mucinous carcinoma (F, H) are categorized as "infiltrative growth" of mucinous carcinoma. G and H, High-power view of (E) and (F), respectively.

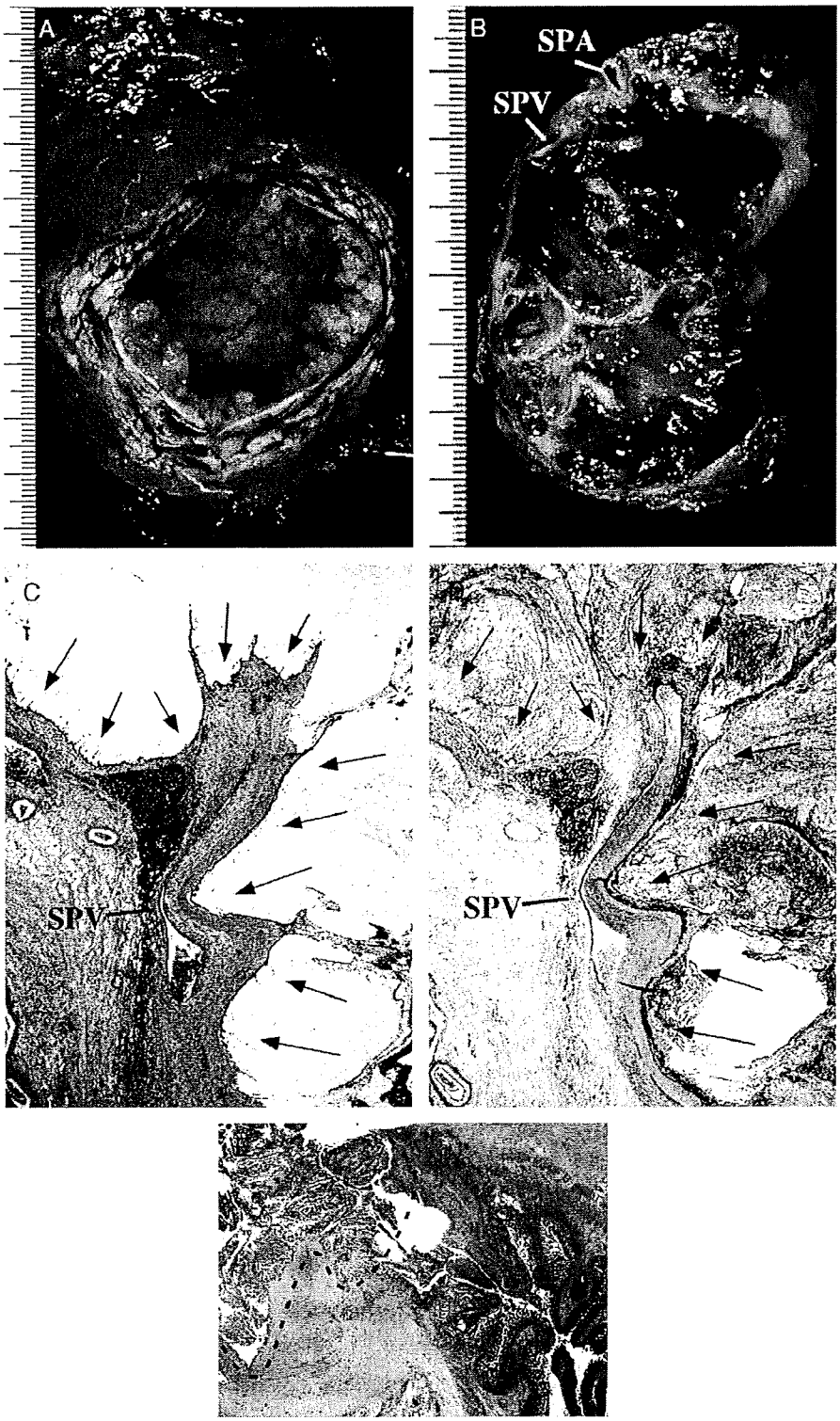

FIGURE 3. Histopathologic features of "expansive growth" in I-IPMC. The pancreatic duct is markedly dilated to a cystlike shape (A, B). Fresh cut view (A) and formalin-fixed cut surface (B) of cystic I-IPMCs. Cystically dilated pancreatic duct is filled with clear mucus and many papillary projections are seen on the inner surface (A). The SPV is compressed (B–D) and its thickened wall is eroded by an enlarged cystic IPMC (arrows) in hematoxylin and eosin stain (C) and elastica stain (D). A fistula has been formed between cystic I-IPMC (dotted line) and duodenum (E).

All of the 24 patients with mucous rupture MI-IPMC survived after surgery.

Expansive growth of ductectatic or cystic IPMN is another characteristic feature of IPMN (Fig. 3). In addition to mucous rupture, an increase of intraductal pressure by hypersecretion of mucus causes marked cystic dilatation of the duct, which continues to grow expansively into extrapancreatic tissue. In some cases, cystic IPMC eventually forms a fistula with surrounding digestive organs (Fig. 3E) or erodes the wall of major blood vessels [portal vein, splenic vein (SPV), superior mesenteric vein (SMV), or splenic artery] (Figs. 3C–E). Such growth and spread are rather passive in contrast to the infiltrative growth that occurs in active invasion and this feature was not associated with poor prognosis, similarly to mucous rupture. IPMC showing expansive growth with loss of the basement membrane of the pancreatic duct in the IPMC is diagnosed as MI-IPMC. If I-IPMC grows expansively, even if it ruptures into the bowel, or even if it erodes a major vessel wall unless cancer cells enter the lumen of the major vessel, it is still regarded as minimal invasion (Table 1). If I-IPMC has this type of growth as predominance, it is corresponded to a kind of pure mucinous carcinoma associated with IPMC.

Although we have not yet experienced intra-abdominal rupture of IPMC, a few cases have been reported.[18] As intra-abdominal rupture was followed by peritoneal dissemination in these reported cases, this type should be distinguished from ordinary IPMN and managed separately as ruptured IPMN.

IC-IPMC was defined as a lesion consisting of IPMN and invasive carcinoma with the predominance of the IPMN component.[12] Such invasive carcinoma exceeds the minimal invasion proposed in Table 1, and shows a continuous transition between invasive carcinoma and intraductal IPMC. In this study, we added new group of cases to the original IC-IPMC category, which had invasive carcinoma apparently originated from IPMN but predominant over the IPMN component. We wanted to compare the prognosis between IC-IPMC and conventional invasive ductal carcinoma of the pancreas in the matched tumor-node-metastasis (TNM) stages.[11]

Statistical Analysis

Comparisons of qualitative variables were performed using the χ^2 test or Fisher exact test. One-way analysis of variance was used to compare the means of 3 or more groups. The postoperative overall and disease-specific survival rates were calculated by the Kaplan-Meier method. Univariate analysis was performed for prognostic factors using the log-rank test. The factors found to be predictive by univariate analysis were subjected to multivariate analysis using the Cox proportional hazards model. Differences at $P < 0.05$ were considered statistically significant. Statistical analyses were performed with SPSS 11.0J software (SPSS Inc, Chicago, IL).

RESULTS

Histopathologic Evaluation of I-IPMC

One hundred and four IPMNs were classified into 27 IPMAs, 11 borderline IPMNs, 15 noninvasive IPMCs, and 51 I-IPMCs according to the WHO classification.[13,15] None of them had an ovarianlike stroma, and all the lesions showed communication with the pancreatic ductal system. I-IPMCs were further divided into 26 MI-IPMCs and 25 IC-IPMCs according to our criteria (Table 1) based on the histopathologic pattern of invasion.

To evaluate the aggressive characteristics of I-IPMC, we examined the invasiveness of I-IPMC. The invasiveness was categorized into 4 patterns: infiltrative growth, mucous rupture, expansive growth, and intra-abdominal rupture (see Materials and Methods). The criterion of minimal invasion was proposed for each corresponding pattern (Table 1), and the representative features are shown in Figures 1 to 3.

Seventeen among 26 patients with MI-IPMC showed infiltrative growth pattern (Fig. 1). Histologic types of the infiltrating cancer cells were tubular adenocarcinoma in 7 patients, mixed tubular adenocarcinoma and mucinous carcinoma in 2 patients, pure mucinous carcinoma in 5 patients, and papillary adenocarcinoma in 3 patients. The average depth of infiltration was 1.5 mm (range from < 1 to 5 mm). None of the 17 patients with a maximum infiltration of 5 mm or less had recurrence with exception of 2 patients, one of them had 2-mm-length infiltration of tubular adenocarcinoma and the other had 2-mm-length infiltration of pure mucinous carcinoma.

The most of the patients with MI-IPMC had mucous rupture and 6 patients had MI-IPMC with mucous rupture as predominant invasive pattern (Fig. 2). Two of them were subcategorized as mucous rupture with cellular component. None of these 6 patients had recurrence.

Expansive growth (Fig. 3) was often observed in cystically growing tumors and 4 patients with MI-IPMC mentioned below showed expansive growth as predominance. In 2 patients with I-IPMC, a fistula was formed between the IPMN and the duodenum. No cancer cells infiltrating the duodenal wall were detected in either case by histologic examination (Fig. 3E). It was suspected that the fistulas were formed by rupture of the expansively growing IPMN into the adjacent duodenum. The lesion was classified as MI-IPMC (expansive growth) in 1 patient, but the other patient had definite invasive cancer in the pancreas tail distant from the fistula, and was therefore diagnosed as having IC-IPMC. Whereas the former patient had no recurrence 107 months after surgery, the latter patient developed local lymph node (LN) metastasis 6 months after surgery and died of the disease. In the other 3 patients with expansive growth of MI-IPMC, the IPMN had grown deeply into the retropancreatic tissue, compressing the wall of the SPV or SMV. In one of them, the tunica media of the SPV was involved without a fistula

TABLE 2. Comparison of Invasive Lesion Between MI-IPMC and IC-IPMC

	MI-IPMC (n = 26)	IC-IPMC (n = 25)	P*
Growth pattern			
Infiltrative growth	17	25	
Mucous rupture or expansive growth as predominance	10†	0	
Vessel or neural invasion	4	25	**< 0.001**
Lymphatic invasion	0	23	**< 0.001**
Venous invasion	2	24	**< 0.001**
Intrapancreatic neural invasion	2	22	**< 0.001**
Extrapancreatic involvement	4	23	**< 0.001**
Serosa	0	4	0.051
Retropancreatic tissue	3‡	21	**< 0.001**
Duodenum	1‡	8	**0.002**
Extrahepatic bile duct	0	3	0.110
Portal venous system	1‡	8	**0.011**
Arterial system	0	1	0.490
Extrapancreatic nerve plexus	0	4	0.051
Invasion to surgical margin	0	4	0.051
Metastasis	0	17	**< 0.001**
Local LN	0	17	**< 0.001**
Distant organs	0	4§	0.051
TNM stage			**< 0.001**
IA	22	1	
IB	0	0	
IIA	4	7	
IIB	0	13	
III	0	0	
IV	0	4§	
Histology of infiltrative growth			
Pap	3	2	
Tub1	7	5	
Tub2	0	8	
Tub+Muc	2	7	
Tub3	0	1	
Muc	5	1	
AS	0	1	

Statistically significant value is in bold characters.

*P value was calculated by χ^2 or Fisher exact test.

†6 patients showed mucous rupture (2 of them showed mucous rupture with cellular component) and 4 patients showed expansive growth (one of them showed infiltrative growth as well).

‡Due to expansive growth.

§One patient with liver metastasis, 3 patients with para-aortic LN metastasis.

AS indicates adenosquamous carcinoma; Muc, mucinous carcinoma; Pap, papillary adenocarcinoma; Tub1, well-differentiated tubular adenocarcinoma; Tub2, moderately differentiated tubular adenocarcinoma; Tub3, poorly differentiated tubular adenocarcinoma.

between tumor and SPV (Figs. 3C–E). These 3 patients did not have postoperative recurrence at 28, 52, and 96 months after surgery, respectively. We thought mucous rupture and expansive growth is dormant invasion, considering its nonaggressive nature, which is characteristic to IPMN.

Comparison of the pathologic characteristics and TNM staging[11] between invasive lesions of MI-IPMCs and IC-IPMCs are summarized in Table 2. Vessel or neural invasion and extrapancreatic involvement were much more common in IC-IPMC than in MI-IPMC. No LN metastasis was observed in patients with MI-IPMC, whereas 17 patients (68%) with IC-IPMC showed LN metastasis. With regard to the histology of the invasive component of the IC-IPMC, most of the patients had tubular adenocarcinoma and only 1 patient had pure mucinous carcinoma. Among 26 patients with MI-IPMC, 9 had tubular adenocarcinoma and 11 had pure mucinous carcinoma.

Prognostic Significance of the Classification of I-IPMC

The median survival period for the 104 patients was 142 months, and the 3, 5, and 10-year overall survival rates were 86%, 78%, and 59%, respectively. There was no statistically significant difference in overall survival among patients with IPMA, borderline IPMN, and noninvasive IPMC ($P = 0.54$). Therefore, they were integrated into noninvasive IPMN for subsequent analysis. The survival rates 3, 5, and 10 years after surgery were 95%, 92%, and 70% for noninvasive IPMN, 95%, 79%, and 79% for MI-IPMC, and 51%, 38%, and 0% for IC-IPMC (Fig. 4A). The disease-specific survival rates after 3, 5, and 10 years were 100%, 100%, and 100% for noninvasive IPMN, 100%, 100%, and 100% for MI-IPMC, and 51%, 38%, and 0% for IC-IPMC (Fig. 4B). Overall and disease-specific survival for MI-IPMC was significantly better than for IC-IPMC ($P < 0.001$), whereas there was no significant difference in overall survival between noninvasive IPMN and MI-IPMC ($P = 0.66$).

Overall survival was compared between I-IPMC and conventional invasive ductal carcinoma of the pancreas during the same period (Figs. 5A–D). The stages of IC-IPMCs were assessed on the basis of size and spread of invasive carcinoma in the lesion, using the International Union against Cancer (UICC) TNM classification,[11] and classified as stage IA, IB, and IIA, stage IIB, and stage III and IV. Between IC-IPMC and conventional invasive ductal carcinoma of the pancreas at each corresponding TNM stage, there was no statistically significant difference in survival rate, though IC-IPMC had a tendency to show a favorable outcome.

Prognostic Factors in I-IPMCs

Clinicopathologic factors possibly affecting the postoperative outcome of I-IPMCs were studied (Table 3). The following variables were significantly related to unfavorable prognosis: presence of jaundice, cancer cells present at the surgical margin except the pancreatic margin, presence of major vascular invasion [portal vein, SMV, SPV, or splenic artery], presence of lymphatic invasion, presence of venous invasion, presence of intrapancreatic neural invasion, presence of LN metastasis, presence of para-aortic LN metastasis, CA19-9 > 300 U/mL, size of invasive cancer > 2 cm, histopathologic diagnosis of IC-IPMC (vs. MI-IPMC), and tubular adenocarcinoma as histologic type of invasive cancer in I-IPMC. Multivariate analysis (backward elimination method) showed that a histopathologic diagnosis of I-IPMC classified as IC-IPMC and

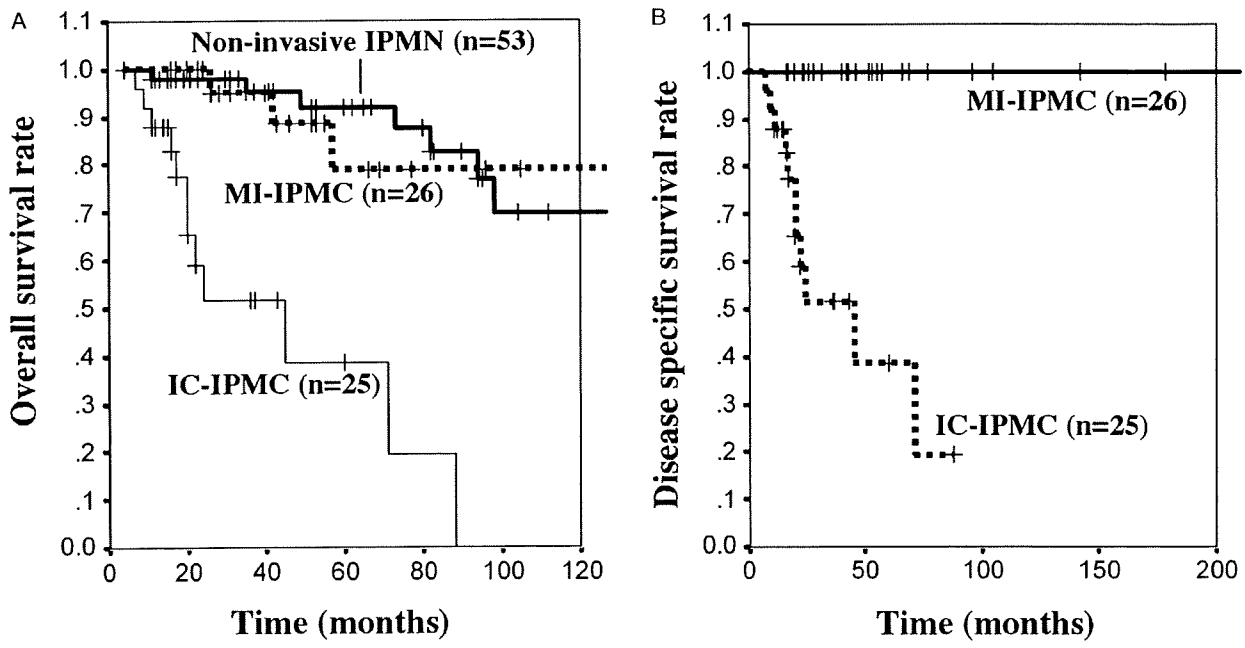

FIGURE 4. Kaplan-Meier survival curves of the 104 patients with IPMNs. A, Overall survival of patients with MI-IPMC was significantly better than that of patients with IC-IPMC ($P<0.001$), whereas no significant difference was found between patients with noninvasive IPMN and those with MI-IPMC ($P=0.66$). B, Disease-specific survival of patients with MI-IPMC was significantly better than that of patients with IC-IPMC ($P<0.001$), with no disease-related death among 26 patients with MI-IPMC during a median follow-up period of 43.4 (13.2 to 210) months.

CA19-9 > 300 U/mL were significant prognostic factors (Table 4).

Postoperative Recurrence of IPMNs

Postoperative recurrence was observed in 15 patients exclusively among those with I-IPMC (Table 5). Two patients with MI-IPMC suffered recurrence of MI-IPMC and invasive cancer in the remnant pancreas 36 and 48 months after surgery, respectively. At initial surgery, both patients had undergone PPPD for IPMNs in the pancreas head with negative surgical margins. The former underwent completion pancreatectomy in a second operation, and pathologic examination revealed another MI-IPMC in the remnant pancreas distant from the site of pancreato-jejunostomy. In the latter patient, recurrence of invasive ductal carcinoma was also found distant from the pancreato-jejunostomy, and additional partial resection of the remnant pancreas was performed. Both patients are currently doing well with no evidence of recurrence 8 and 20 months after the second operation, respectively. The remaining 13 recurrences were observed in patients with IC-IPMC. The site of recurrence was local (remnant pancreas) in 2 patients, LN in 2 patients, the lung in 1 patient, the liver in 4 patients, and peritoneal dissemination in 4 patients (Table 5). The time interval between surgery and recurrence was less than 20 months in all cases, with an especially short duration of 6.15 ± 0.82 months for patients with peritoneal dissemination.

Analysis of the Pancreatic Surgical Margin

Intraoperative frozen section analysis of the pancreas margin was performed in 96 patients, and 17 patients needed additional pancreatic resection owing to the confirmed or suspected presence of cancer cells at the pancreatic surgical margin (Table 5). Additional resection was performed more frequently in patients with MI-IPMC and IC-IPMC than in those with noninvasive IPMN, regardless of IPMN size ($P = 0.007$). The final pancreatic margin status was negative in 75 patients, positive for IPMA in 25, borderline IPMN in 2, noninvasive IPMC in 1, and invasive carcinoma in 1.

DISCUSSION

Many groups have investigated the malignant potential of IPMNs,[4,6,16,20,22–24] and the recent consensus is that its aggressiveness is dependent on the presence of invasive cancer, the extent of cancer invasion, and the biologic characteristics of the cancer cells.[2,3,8,10,14,15] However, no sufficient pathologic and presurgical staging system has yet been established for evaluating the malignant potential of I-IPMC. In this study, we examined 104 IPMNs surgically resected at the same hospital and proposed histopathologic criteria for classification of I-IPMC. I-IPMC shows heterogeneous features, which reflect the presence of heterogeneous cancer types with different biologic behaviors. Therefore, the criteria of MI-IPMC should differ in accordance with each histopathologic pattern of invasion. Our proposed

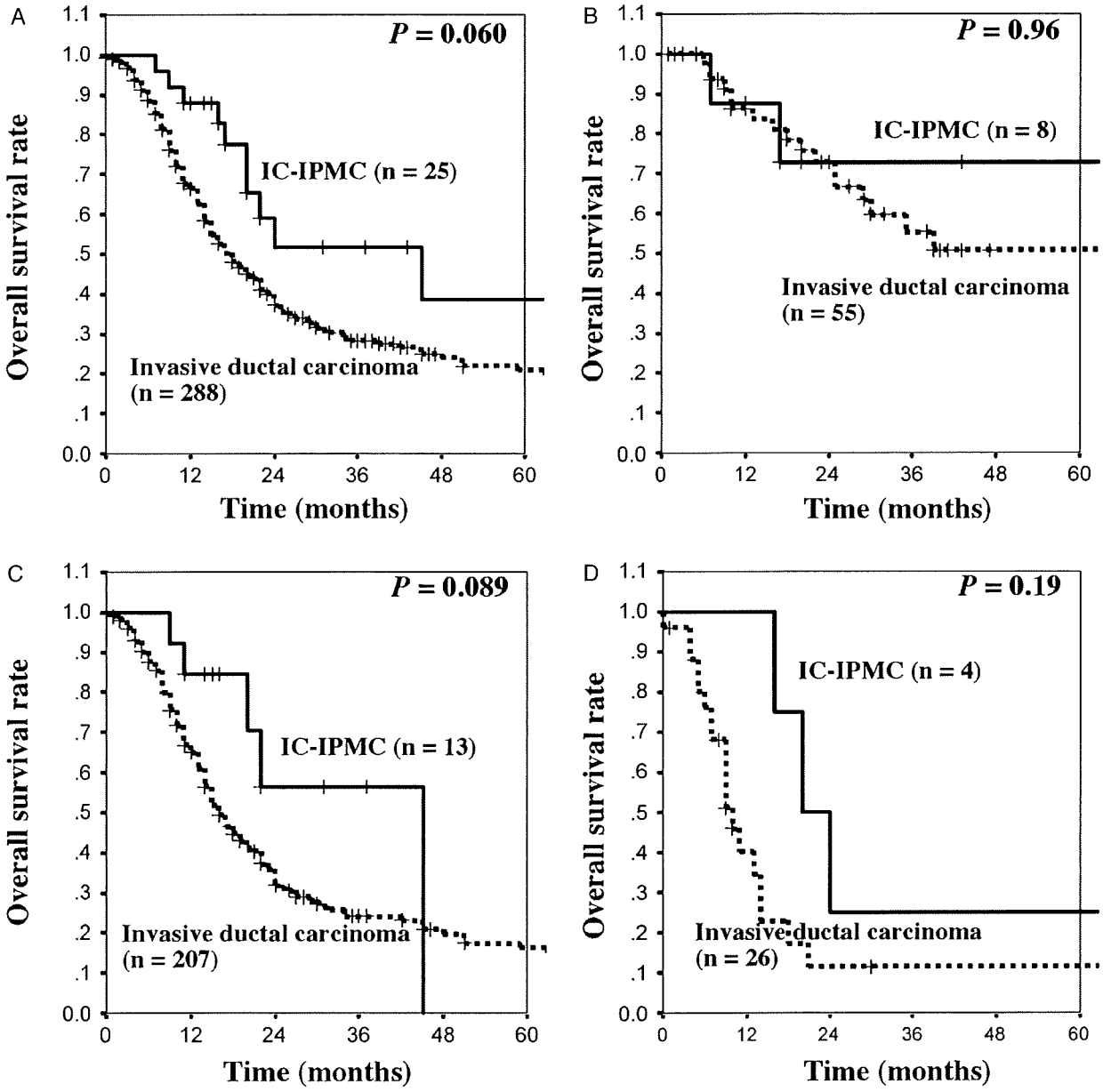

FIGURE 5. Kaplan-Meier survival curves of the 25 patients with IC-IPMC and the 288 patients with invasive ductal carcinoma of the pancreas. Comparison of overall survival of the patients with IC-IPMC and that of patients with conventional invasive ductal carcinoma at all stages (A), and in stage IA, IB, and IIA (B), stage IIB (C), and stage III and IV (D). Although the patients with IC-IPMC tended to have a better outcome than those with conventional invasive ductal carcinoma at each corresponding stage, the difference was not statistically significant.

criteria of invasiveness were successful in categorizing IPMCs in our series into noninvasive IPMC, MI-IPMC, and IC-IPMC. Patients with IC-IPMC had a significantly worse outcome than those with MI-IPMC. However, there was no difference in postoperative outcome between patients with MI-IPMC and those with noninvasive IPMC. This is the first report to propose practical criteria for MI-IPMC that can separate early-stage nonaggressive I-IPMC from total I-IPMC. Discrimination between MI-IPMC and IC-IPMC can provide important information for predicting the postoperative outcome of patients with IPMNs and also for deciding additional clinical management.

When IC-IPMCs were staged according to the size and spread of an invasive carcinoma component, the survival curve showed a similar decline to that of conventional invasive ductal carcinomas of the corresponding TNM stage, suggesting that it is the invasive

TABLE 3. Prognositic Factors of I-IPMCs in Univariate Analysis

Variables	n	Survival Rate 1 y	3 y	5 y	P (Log-rank Test)
Sex					0.262
M	27	96.3	80.4	80.4	
F	24	91.7	70.4	41.5	
Age (y)					0.082
≤70	33	100.0	80.6	72.6	
> 70	18	83.3	67.3	46.2	
Tumor location					0.937
Ph included	33	93.9	72.4	60.8	
Ph excluded	18	94.4	84.0	67.2	
Tumor distribution					0.821
Confined in 1 segment	35	91.4	75.7	63.2	
Diffuse (≥2 segments)	16	100.0	76.6	63.8	
PV resection					0.471
+	5	80.0	53.3	53.3	
–	46	95.7	78.0	63.0	
Chief complaint*					0.225
+	28	92.9	65.6	49.2	
–	23	95.6	90.0	81.0	
Jaundice					**0.011**
+	6	83.3	27.8	0.0	
–	45	95.6	80.7	66.2	
IPMN type					0.571
MPD or mixed	40	97.5	76.2	59.0	
BD	11	81.8	72.7	72.7	
MPD diameter					0.422
≤8 mm	31	90.3	75.4	52.2	
> 8 mm	20	100.0	75.6	75.6	
Additional resection of pancreas†					0.864
+	14	92.9	63.7	63.7	
–	37	94.6	78.4	60.3	
Surgical margin (except for pancreas margin)‡					**< 0.001**
+ or ±	4	75.0	0.0	0.0	
–	47	95.7	84.4	69.1	
Major vascular invasion (SMV, SPV, PV, or SPA)					**0.009**
+	10	90.0	48.2	0.0	
–	41	95.1	82.0	71.2	
Lymphatic invasion					**< 0.001**
+	23	87.0	44.7	22.4	
–	28	100.0	95.5	81.7	
Venous invasion					**0.006**
+	26	88.5	53.5	42.8	
–	25	100.0	94.4	78.0	
Intrapancreatic neural invasion					**< 0.001**
+	24	87.5	52.7	39.5	
–	27	100.0	94.4	78.4	
Local LN metastasis					**< 0.001**
+	18	88.9	47.1	23.5	
–	33	97.0	88.9	76.4	
Para-aortic LN metastasis					**< 0.001**
+	3	100.0	0.0	0.0	
–	48	93.8	32.3	67.4	
CEA (ng/mL)					0.455
≤5	35	94.3	83.4	64.3	
> 5	16	93.8	58.4	58.4	
CA19-9 (U/mL)					**< 0.001**
≤300	40	97.5	84.5	79.8	
> 300	11	81.8	40.9	0.0	
IPMN size (mm)					0.552
≤40	15	93.3	70.2	43.9	
> 40	36	94.4	78.2	72.2	
IPMN size (mm)					0.762
≤70	33	90.9	74.8	60.0	
> 70	18	100.0	77.0	66.0	
Size of invasive lesion (mm)					0.001
≤20	32	96.9	88.7	75.1	
> 20	19	89.5	48.7	32.4	
Pathologic diagnosis					**< 0.001**
MI-IPMC	26	100.0	94.7	78.6	
IC-IPMC	25	88.0	50.6	38.0	
Histology of invasive cancer in I-IPMC					
Tubular adenocarcinoma§	29	89.7	61.8	54.1	0.065
Pure mucinous carcinoma	12	100.0	100.0	57.1	
Tubular adenocarcinoma§	29	89.7	61.8	54.1	**0.010**
Nontubular adenocarcinoma	22	100.0	94.4	71.6	
Pure mucinous carcinoma	12	100.0	100.0	57.1	0.162
Nonpure mucinous carcinoma	39	92.3	68.6	62.9	
Past history of another cancer in other organs					0.316
+	10	90.0	72.0	54.0	
–	41	95.1	79.8	63.3	
TNM stage					
MI-IPMC	26	100.0	94.7	78.6	**0.040**
Stages IA, IB, and IIA	8	87.5	70.0	70.0	0.42
Stage IIB	13	84.6	56.4	0.0	0.82
Stages III and IV	4	100.0	25.0	25.0	

Statistically significant value is in bold characters.
*Diabetes mellitus exacerbation and jaundice included.
†Due to existence of neoplastic cells in pancreas margin in frozen section analysis.
‡Presence of invasive carcinoma cells in the stroma.
§Mixed tubular adenocarcinoma and mucinous carcinoma were included.
BD indicates branch duct; MPD, main pancreatic duct; Ph, pancreatic head; PV, portal vein.

carcinoma rather than IPMN itself that determines the prognosis.

In our series, none of the patients with MI-IPMCs showed LN metastasis, whereas the patients with IC-IPMCs had a high rate (68%) of LN metastasis. This finding implies that complete resection of a lesion without LN dissection may be sufficient for the treatment of MI-IPMC, whereas radical pancreatectomy with LN dissection is indicated for IC-IPMC. In this context, preoperative distinction between MI-IPMCs and IC-IPMCs is clinically very important.

TABLE 4. Multivariate Analysis of Effects of Clinicopathologic Factors on Postoperative Survival of I-IPMC

	Hazard Ratio	95% Confidence Interval	P*
IC-IPMC (vs. MI-IPMC)	7.1	1.9-26.5	< 0.001
CA19-9 > 300 (U/mL)	4.4	1.4-13.8	0.010

*P value was calculated by Cox hazards model (backward elimination method).

TABLE 5. Pancreatic Margin Status and the Recurrence of IPMNs After Surgery

	IPMA or Borderline IPMN (n = 38)	Noninvasive IPMC (n = 15)	MI-IPMC (n = 26)	IC-IPMC (n = 25)	Total (n = 104)	*P*
Additional pancreas resection*	3	0	5	9	17	0.007†
Final margin status						0.071
Negative	26	10	20 (2)	19 (11)	75	
IPMA	12	5	5	3 (1)‡	25	
Borderline IPMN	0	0	1	1	2	
Noninvasive IPMC	0	0	0	1	1	
Invasive cancer	0	0	0	1 (1)§	1	
Recurrence						
MI-IPMC (in the remnant pancreas)	0	0	1	0	1	
Invasive cancer (in the remnant pancreas)	0	0	1	0	1	
Local recurrence of invasive cancer	0	0	0	2	2	
Local LN	0	0	0	2	2	
Distant metastasis (lung or liver)	0	0	0	5	5	
Peritoneal dissemination	0	0	0	4	4	
Total	0	0	2	13	15	

*Due to the presence of neoplastic cells in the pancreatic surgical margin in the frozen section analysis.
†Comparison between noninvasive IPMN and I-IPMC.
‡Liver metastasis.
§Local recurrence, numbers in the parentheses denotes the number of patients who developed recurrence after the operation.

Another significant finding was a predominantly high recurrence rate among patients with IC-IPMC (52%), compared with 2.5% for patients with noninvasive IPMN or MI-IPMC. In the latter group, recurrence was observed in the remnant pancreas distant from the cut end, suggesting that IPMC occurred multifocally. Although this recurrence rate is not as high as that reported previously,[4] careful follow-up seems to be necessary after surgery, especially in patients with IC-IPMCs.

Our criteria are not contradictory to the previous studies, in which the postoperative outcome of I-IPMC with pure mucinous carcinoma (colloid carcinoma) was better than that of patients with I-IPMC with tubular adenocarcinoma in the invasive lesion.[1,24] Tubular adenocarcinoma shows active infiltrative growth similar to conventional pancreatic ductal adenocarcinoma, suggesting that it rapidly grows and progresses into advanced cancer. In fact, tubular adenocarcinoma occurred at a higher rate in IC-IPMC than in MI-IPMC, and was an unfavorable prognostic factor ($P = 0.010$; Table 3). It has been reported that mucinous carcinoma associated with IPMN or mucinous cystic tumor has a better outcome than conventional ductal carcinoma. According to Adsay's criteria (a carcinoma with more than 80% of mucinous carcinoma is defined as pure mucinous carcinoma),[1] 12 I-IPMCs were diagnosed as pure mucinous carcinoma associated with IPMC in our series, which contained 11 MI-IPMC (5 with infiltrative growth of pure mucinous carcinoma, 2 with predominantly mucous rupture with cellular component, and 4 with expansive growth) and 1 IC-IPMC. Among these 12 patients with pure mucinous carcinoma associated with IPMC, 1 patient with MI-IPMC with infiltrative growth and 1 patient with IC-IPMC had recurrence of the carcinoma. Although 12 patients had the recurrent cancers and 10 of them died among 29 patients of I-IPMCs with tubular adenocarcinoma (8 in MI-IPMC and 21 in IC-IPMC). Patients with pure mucinous carcinoma as histologic type of invasive cancer tended to have better prognosis than patients with tubular adenocarcinoma as invasive cancer ($P = 0.065$; Table 3). Our study also suggested that some mucinous carcinoma has aggressive behavior. The prognosis of mucinous carcinoma in the other organs such as colon, has been reported to be worse than the ordinary adenocarcinoma, especially worse for mucinous carcinoma with rich cellular component.[17,21] In ductal carcinoma of the pancreas, mixed mucinous carcinoma with other histologic types of carcinoma (usually tubular adenocarcinoma) shows bad prognosis comparable with the other types of conventional ductal adenocarcinoma.[7,14] In this situation, it is desired that a diagnostic criterion is established to distinguish aggressive and nonaggressive mucinous carcinoma correctly. In this study, addition to the classification of tubular adenocarcinoma of the I-IPMC into aggressive and nonaggressive state, we also classified mucinous carcinoma relevant to clinical behavior based on the invasiveness and cellularity. Compared with mucous rupture, more aggressive mucinous carcinoma shows massive invasion with much more cancer cells floating and proliferating in mucus lakes, and is often accompanied by partial invasion of tubular adenocarcinoma.

Lymphatic, venous, and intrapancreatic neural invasion were frequently observed in IC-IPMC (Table 2) and were significant prognostic factors in I-IPMC (Table 3). In this study, we tried to select early-stage I-IPMC with nonaggressive characters from I-IPMCs with such worse prognostic factors. We successfully selected it by categorizing the infiltrating depth of cancer cells, which included lymphatic, venous, and/or neural invasion. Indeed, all the patients with MI-IPMC having vessel or neural invasion within 5-mm length from IPMC duct

showed good postoperative outcome. In addition, lymphatic, venous, and intrapancreatic neural invasion were not significant variables for the prognosis in multivariate analysis (Table 4).

The present results suggest that IC-IPMC (not MI-IPMC) should be currently paid attention as I-IPMC with aggressive characteristics. In this situation, preoperative detection of IC-IPMC can be beneficial for selecting the most ideal operative procedure, especially on considering additional LN dissection. We are now investigating possible criteria for classifying these cancers preoperatively, and our findings suggest that it may be feasible to use radiologic data for this purpose. Multidetector row computed tomography was found to be useful to distinguish IC-IPMC from MI-IPMC and noninvasive IPMNs with more than 80% sensitivity and 100% specificity in the study using 123 patients with IPMNs (manuscript in preparation).

In future, we would like to test our criteria using another large series of samples or in a prospective study, to obtain more watertight pathologic criteria for classification of I-IPMC.

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REFERENCES

1. Adsay NV, Merati K, Nassar H, et al. Pathogenesis of colloid (pure mucinous) carcinoma of exocrine organs. *Am J Surg Pathol.* 2003; 27:571–578.
2. Adsay NV, Merati K, Basturk O, et al. Pathologically and biologically distinct types of epithelium in intraductal papillary mucinous neoplasms. Delineation of an 'intestinal' pathway of carcinogenesis in the pancreas. *Am J Surg Pathol.* 2004;28:839–848.
3. Biankin AV, Kench JG, Dijkman FP, et al. Molecular pathogenesis of precursor lesions of pancreatic ductal adenocarcinoma. *Pathology.* 2003;35:14–24.
4. Chari ST, Yadav D, Smyrk TC, et al. Study of recurrence after surgical resection of intraductal papillary mucinous neoplasm of the pancreas. *Gastroenterology.* 2002;123:1500–1507.
5. Cho KR, Vogelstein B. Genetic alterations in the adenoma-carcinoma sequence. *Cancer.* 1992;70:1727–1731.
6. D'Angelica M, Brennan MF, Suriawinata AA, et al. Intraductal papillary mucinous neoplasms of the pancreas: an analysis of clinicopathologic features and outcome. *Ann Surg.* 2004;239:400–408.
7. Fukushima N, Mukai K, Kanai Y, et al. Intraductal papillary tumors and mucinous cystic tumors of the pancreas. *Hum Pathol.* 1997;28:1010–1017.
8. Furukawa T, Klöppel G, Volkan Adsay N, et al. Classification of types of intraductal papillary-mucinous neoplasm of the pancreas: a consensus study. *Virchows Arch.* 2005;447:794–799.
9. Hiraoka N, Onozato K, Kosuge T, et al. Prevalence of FOXP3$^+$ regulatory T cells increases during the progression of pancreatic ductal adenocarcinoma and its precursor lesions. *Clin Cancer Res.* 2006;12:5423–5434.
10. Hruban RH, Takaori K, Klimstra DS, et al. An illustrated consensus on the classification of pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasms. *Am J Surg Pathol.* 2004;28:977–987.
11. International Union Against Cancer (UICC). *TNM Classification of Malignant Tumors.* 6th ed. New York, NY: Wiley-Liss; 2002.
12. Japan Pancreas Society. *Classification of Pancreatic Cancer.* 2nd ed. Tokyo, Japan: Kanehara; 2003.
13. Klöppel G, Solcia E, Longnecker DS, et al. *Histological Typing of Tumors of the Exocrine Pancreas, World Health Organization International Histological Classification of Tumors.* 2nd ed. Berlin, Germany: Springer; 1996.
14. Klöppel G, Hruban RH, Longnecker DS, et al. Ductal adenocarcinoma of the pancreas. In: Hamilton SR, Aaltonen LA, eds. *Pathology and Genetics. Tumours of the Digestive System. World Health Organization Classification of Tumours.* Lyon, France: IARC Press; 2000:221–230.
15. Longnecker DS, Adler G, Hruban RH, et al. Intraductal papillary-mucinous neoplasms of the pancreas. In: Hamilton SR, Aaltonen LA, eds. *Pathology and Genetics. Tumours of the Digestive System. World Health Organization Classification of Tumours.* Lyon, France: IARC Press; 2000:237–240.
16. Maire F, Hammel P, Terris B, et al. Prognosis of malignant intraductal papillary mucinous tumours of the pancreas after surgical resection. Comparison with pancreatic ductal adenocarcinoma. *Gut.* 2002;51:717–722.
17. Minsky BD, Mies C, Rich TA, et al. Colloid carcinoma of the colon and rectum. *Cancer.* 1987;60:3103–3112.
18. Mizuta Y, Akazawa Y, Shiozawa K, et al. Pseudomyxoma peritonei accompanied by intraductal papillary mucinous neoplasm of the pancreas. *Pancreatology.* 2005;5:470–474.
19. Nakagohri T, Asano T, Kenmochi T, et al. Long-term surgical outcome of noninvasive and minimally invasive intraductal papillary mucinous adenocarcinoma of the pancreas. *World J Surg.* 2002;26:1166–1169.
20. Raimondo M, Tachibana I, Urrutia R, et al. Invasive cancer and survival of intraductal papillary mucinous tumors of the pancreas. *Am J Gastroenterol.* 2002;97:2553–2558.
21. Ronnett BM, Zahn CM, Kurman RJ, et al. A Clinicopathologic analysis of 109 cases with emphasis on distinguishing pathologic features, site of origin, prognosis, and relationship to "pseudomyxoma peritonei". *Am J Surg Pathol.* 1995;19:1390–1408.
22. Salvia R, Fernandez-del Castillo C, Bassi C, et al. Main-duct intraductal papillary mucinous neoplasms of the pancreas: clinical predictors of malignancy and long-term survival following resection. *Ann Surg.* 2004;239:677–678.
23. Shimada K, Sakamoto Y, Sano T, et al. Invasive carcinoma originating in an intraductal papillary mucinous neoplasm of the pancreas: a clinicopathologic comparison with a common type of invasive ductal carcinoma. *Pancreas.* 2006;32:281–287.
24. Sohn TA, Yeo CJ, Cameron JL, et al. Intraductal papillary mucinous neoplasms of the pancreas: an updated experience. *Ann Surg.* 2004;239:788–789.
25. Suzuki Y, Atomi Y, Sugiyama M. Cystic neoplasm of the pancreas: a Japanese multi-institutional study of intraductal papillary mucinous tumor and mucinous cystic tumor. *Pancreas.* 2004;28:241–246.
26. Wells M, Östör AG, Crum CP, et al. Epithelial tumours. In: Tavassoli FA, Devilee P, eds. *Pathology and Genetics of Tumours of the Breast and Female Genital Organs. World Health Organization Classification of Tumours.* Lyon, France: IARC Press; 2003: 262–269.
27. Yamao K, Ohashi K, Nakamura T, et al. The prognosis of intraductal papillary mucinous tumors of the pancreas. *Hepatogastroenterology.* 2000;47:1129–1134.

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Prognosis of Perihilar Cholangiocarcinoma: Hilar Bile Duct Cancer versus Intrahepatic Cholangiocarcinoma Involving the Hepatic Hilus

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Background: Clinically hepatobiliary resection is indicated for both hilar bile duct cancer (BDC) and intrahepatic cholangiocarcinoma involving the hepatic hilus (CCC). The aim of this study was to compare the long-term outcome of BDC and CCC.

Methods: Between 1990 and 2004, we surgically treated 158 consecutive patients with perihilar cholangiocarcinoma. The clinicopathological data on all of the patients were analyzed retrospectively.

Results: The overall 3-year survival rate, 5-year survival rate, and median survival time for BDC patients were 48.4%, 38.4 %, and 33.7 months, respectively, and 35.8%, 24.5 %, and 22.7 months, respectively, in CCC patients (P = .033).

On multivariate analysis, three independent factors were related to longer survival in BDC patients: achieved in curative resection with cancer free margin (R0) (P = .024, odds ratio 1.862), well differentiated or papillary adenocarcinoma (P = .011, odds ratio 2.135), and absence of lymph node metastasis (P < .001, odds ratio 3.314). Five factors were related to longer survival in CCC patients: absence of intrahepatic daughter nodules (P < .001, odds ratio 2.318), CEA level ≤2.9 ng/mL (P = .005, odds ratio 2.606), no red blood cell transfusion requirement (P = .016, odds ratio 2.614), absence or slight degree of lymphatic system invasion (P < .001, odds ratio 4.577), and negative margin of the proximal bile duct (P = .003, odds ratio 7.398).

Conclusions: BDC and CCC appear to have different prognoses after hepatobiliary resection. Therefore, differentiating between these two categories must impact the prediction of postoperative survival in patients with perihilar cholangiocarcinoma.

Key Words: Hilar bile duct cancer—Intrahepatic cholangiocarcinoma—Hepatobiliary resection.

Hilar cholangiocarcinoma remains a challenging disease, and the prognosis is often dismal, even after aggressive surgery including hepatobiliary resection with caudate lobectomy.[1] Previous reports have included a limited number of resected cases, and reports of large, single-center studies are not common.[2–13]

Based on the anatomical origin of the tumor, hilar cholangiocarcinoma and perihilar cholangiocarcinoma are potentially divisible into two categories: hilar bile duct cancer (BDC) and intrahepatic cholangiocarcinoma involving the hepatic hilus (CCC). BDC originates in the epithelium of the common hepatic, right or left hepatic duct, whereas CCC

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originates in the intrahepatic bile duct or bile ductules.

In the clinical setting, curative resection for both BDC and CCC involves hepatobiliary resection with regional lymphadenectomy. Many previous studies have treated BDC and CCC as the same entity; thus, the clinicopathological differences between BDC and CCC remain unclear, and the clinical usefulness of differentiating between these two groups has not been elucidated.

We have distinguished between BDC and CCC based on pathology and have collected the clinicopathological data since the 1980s. Thus, a review of these patients' data is crucial for determining future strategies.

The aims of this study were to review the long-term outcome of major hepatobiliary resections done over the last 15 years for BDC and CCC using a similar treatment strategy in a single center and to characterize the prognostic factors affecting the long-term outcome for each group to clarify the differences between groups.

PATIENTS AND METHODS

Between January 1, 1990 and December 31, 2004, 225 patients were admitted to our department with a tentative diagnosis of perihilar cholangiocarcinoma. The following patients were excluded: patients who did not undergo laparotomy because of highly advanced disease or poor hepatic functional reserve during the preoperative workup, those in whom resection was not possible due to locally advanced status or dissemination, patients who had a hilar bile duct resection or a minor hepatectomy, and patients with gallbladder cancer or benign biliary stricture based on postoperative pathology. Thus 158 patients, consisting of 99 patients (62.7%) with hilar bile duct cancer (BDC) and 59 patients (37.3%) with intrahepatic cholangiocarcinoma involving the hepatic hilus (CCC), treated with major hepatobiliary resection were enrolled in this study (Table 1). The patient population consisted of 52 women and 106 men, with a median age of 65 years (range, 33–83 years).

The medical records that had been collected, including the hospital charts, operation records, and pathology reports, were analyzed retrospectively.

Our standard management strategies and surgical procedures have been described previously.[14] Briefly, after a preoperative imaging diagnosis of tumor extension was made, biliary drainage was done to ensure that the patient recovered from cholestatic liver damage, if necessary. To induce compensatory hypertrophy of the future remnant liver, preoperative portal vein embolization (PVE)[15,16] for the liver segment to be resected was done if the estimated resection volume exceeded 50–55% of the whole liver; 71 patients (44.9%) underwent PVE.

All patients underwent major hepatobiliary resection with a hepatectomy involving two or more sectors and systematic lymphadenectomy of the nodes located at the hepatoduodenal ligament, the upper part of the retropancreatic nodes, and the celiac nodes, as well as skeletonization of the hepatic hilus. Operative mortality included both death within 30 days of surgery and all in-hospital death. Morbidity included all postoperative complications that affected the outcome or lengthened the hospital stay. The surgical procedures are summarized in Table 2.

Histopathological Evaluation, Pathological Diagnosis, and Staging

First, the extrahepatic bile duct was incised longitudinally from the distal to the proximal margin. The anatomical orientation of the individual vessels and the surgical margins of the resected specimen were assessed macroscopically. The en bloc dissected lymph nodes were classified according to the anatomical location. Both the proximal and distal margins of the bile duct were routinely evaluated using intraoperative frozen section.

The resected specimen was fixed in 10% formalin, after that multiple, 5-mm thick, thin slice sections of the resected specimen were prepared in alignment with the computed tomography (CT) plane. In every section, the biliary anatomy was identified in relation to the vasculature. Then, sagittal, thin slice sections every 3 to 5 mm were added to precisely determine tumor extension around the hepatic hilum. Distal tumor extension was clarified based on the serial perpendicular sections to the longitudinal axis of the distal bile duct. In every case, histological tumor extension was investigated in 20 to 40 sections, and lymph node involvement was independently evaluated.

The criteria used to discriminate between BDC and CCC depended primarily on the location of the main tumor, as is schematically shown in Fig. 1. BDC was defined as a tumor originating in the upper common, right or left hepatic duct. Representative cases of BDC and CCC on the slice section of resected specimen are illustrated in Fig. 2. To evaluate the origin or the dominant spatial location of the tumor, hematoxylin-eosin staining was routinely used; elastica stain was additionally used to delineate the elastic

TABLE 1. *Patient characteristics and preoperative variables*

Variable	BDC (n = 99)	CCC (n = 59)	P value
Age (years)	64, [33–83]	66 [34–82]	
Gender (men / women)	69/30	37/22	
Preoperative biliary drainage (performed)	77 (78%)	16 (27%)	<.001
ICGR15 (%)	8.4 [0.8–48.1]	7.1 [0.2–63.2]	
CEA (ng/mL)	2.5 [0.7–22.1]	2.9 [0.8–560]	
CA19-9 (U/mL)	101 [1–14,750]	306 [1–256,800]	.006

[range].
ICGR15 indicates indocyanine green retention value at 15 minutes; CEA, carcinoembrionic antigen; CA19-9, carbohydrate antigen 19-9.

TABLE 2. *Surgical procedures and operative variables*

	BDC (n = 99)	CCC (n = 59)	P value
Type of hepatectomy			
Left hepatectomy	42 (42%)	29 (49%)	
Left trisectionectomy	5 (5)	9 (15)	
Central bisectionectomy	1 (1)	1 (2)	
Right hepatectomy	49 (49)	18 (31)	
Right trisectionectomy	2 (2)	2 (3)	
with PD	10 (10)	0	.012
with PV	18 (18)	14 (24)	
with PVE	53 (54)	18 (31)	.005
Right-sided hepatectomy	51 (52)	20 (34)	.033
Operation time (minutes)	655 [302–1125]	616 [372–950]	.051
Intraoperative blood loss (g)	1670 [446–5087]	1574 [445–7530]	
Red blood cell transfusion performed	34 (34)	26 (44)	
Postoperative morbidity	51 (52)	32 (54)	
In-hospital mortality	0 (0)	2 (3)	.066

[range]
PD, pancreatoduodenectomy; PV, resection and reconstruction of the portal vein; PVE, portal vein embolization prior to the resectional surgery.
Percentages are described in parentheses.

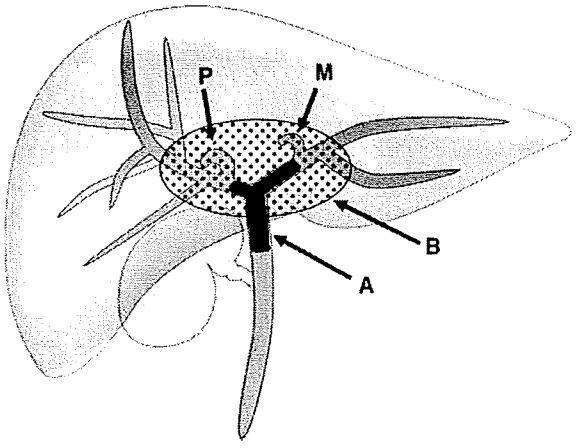

FIG. 1. Tumor origin for differentiating hilar bile duct cancer (BDC) and intrahepatic cholangiocarcinoma involving the hepatic hilus (CCC). Histopathologically, tumors thought to be originated in the black pasted area (**A**) were defined as BDC, and also tumors thought to be originated in the area with dot spots (**B**) were defined as CCC. P, right posterior sectional branch of the bile duct; M, left medial sectional branch of the bile duct.

fibers of the hepatic hilum and the intrahepatic Glisson's capsule in difficult cases of differentiating BDC from CCC. Namely, we can estimate special tumor domination whether inside or outside of hilar plate by the aid of elastica stain.

Macroscopically, the BDC tumors were classified as being polypoid and nodular or infiltrating. The CCC tumors were classified by the pathologists as being mass forming or non mass forming (periductal infiltrating or intraductal growth) type[17] on the plane of the thin slice section.

Pathological TNM classification was determined according to the criteria of the International Union Against Cancer (UICC) (sixth edition),[18] using the chapter dealing with extrahepatic bile duct cancer for BDC and that dealing with liver cancer for CCC. During the study period, the histopathological diagnoses were recorded and accumulated, then reviewed by the pathologists for this paper.

Follow-Up

All patients were followed at our outpatient clinic, where chest x-rays, abdominal ultrasound, CT, and the measurement of CEA and CA19-9 levels was done every 3–6 months after surgery. In principle, postoperative

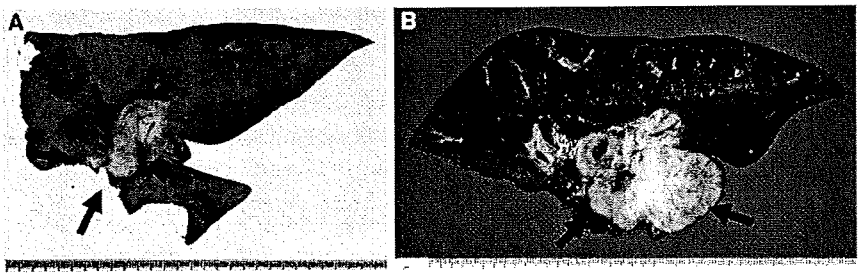

FIG. 2. Representative case of hilar bile duct cancer (A) and intrahepatic cholangiocarcinoma involving the hepatic hilus (B) on the slice section of resected specimen. Arrows indicate tumor.

adjuvant therapy such as chemotherapy, radiotherapy, or chemoradiotherapy was not adopted until tumor recurrence was definitively diagnosed.

Statistical Analysis

The results are expressed as median values, with the respective ranges indicated within square brackets. The relationship between the postoperative morbidity and the dichotomous variables was evaluated by chi-square analysis or Fisher's test, whichever was appropriate. The statistical significance of continuous variables was determined using the Mann-Whitney test. Patient survival was calculated using the Kaplan-Meier method, including deaths from all causes. Univariate comparisons of the survival curves were performed using the log-rank test. Multivariate regression analysis (backward elimination method) was performed using the Cox proportional hazards model,[19] and variables associated with $P < .10$ were entered into the final model. Results were considered significant when the P values were less than .05. The statistical analyses were performed using a statistical analysis software package (SPSS 11.5, SPSS Inc. Chicago, IL).

RESULTS

The patients' overall 1-, 3-, and 5-year survival rates were 81.0%, 43.7%, and 33.4%, respectively. The median survival was 28.4 (4.1–187.1) months, and the median follow-up time was 25.2 (4.1–187.1) months. Ninety-seven patients died of tumor recurrence, and two patients died without evidence of tumor recurrence. The remaining 59 patients are currently alive; 12 have recurrence, and 47 have no sign of recurrence at the time of writing.

The patient characteristics and preoperative variables are summarized in Table 1. The six clinicopathological variables were compared. Preoperative biliary drainage was performed significantly more frequently in BDC patients ($P < .001$). Serum CA19-9 levels were significantly higher in CCC patients ($P = .006$). There were no significant differences in other variables between BDC and CCC patients. There were no in-hospital deaths in the BDC group, but two patients with CCC died in hospital (CCC mortality rate, 3.4%; overall mortality rate, 1.3%). Eighty-three patients (52.5%) developed postoperative morbidity. There were no statistically significant differences in mortality or morbidity between the two groups (Table 2).

The overall 1-, 3-, 5-year survival rates and median survival time of BDC patients were 87.9%, 48.4%, 38.4 %, and 33.7 months, respectively. The overall 1-, 3-, 5-year survival rates and median survival time of CCC patients were 69.5%, 35.8%, 24.5 %, and 22.7 months, respectively. There was a significant difference in the overall survival between BDC and CCC patients ($P = .033$) (Fig. 3). Figures 4**A** and 4**B** show the survival curves of BDC and CCC patients by UICC staging. Significant differences were noted between stages I and II ($P = .0023$), stages I and III ($P = .0453$), and stages I and IV ($P = .0006$) in BDC patients (Fig. 4**A**). Significant differences were also noted between stages I and IV ($P = .0039$), stages II and IV ($P = .0112$), and stages III and IV ($P = .0285$) in CCC patients (Fig. 4**B**). For any given stage, there was no significant difference in survival between BDC and CCC patients: stage I ($P = .5016$), II ($P = .3316$), III ($P = .9584$), and IV ($P = .1387$).

The surgical procedures and operative variables are summarized in Table 2. Hepatopancreatoduodenectomy (HPD)[20] ($P = .012$), PVE ($P = .005$), and right-sided hepatectomy ($P = .033$) were performed significantly more frequently in BDC patients. There were no other significant differences in the surgical procedures or operative variables between the BDC and CCC patients.

The 11 histopathological variables were compared (Table 3). Well differentiated or papillary adenocarcinoma ($P = .034$) and positive proximal ($P = .046$)

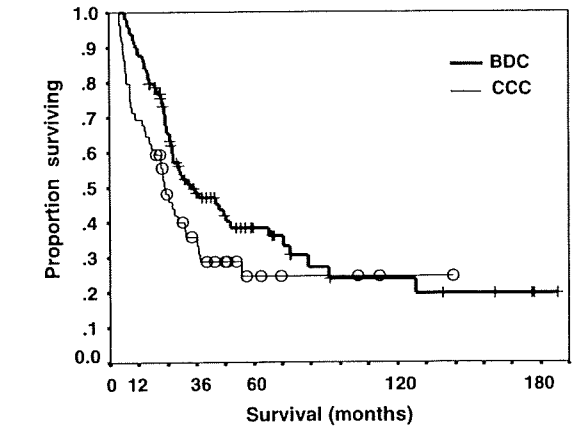

FIG. 3. The survival curves for hilar bile duct cancer (BDC) patients and intrahepatic cholangiocarcinoma involving the hepatic hilus (CCC) patients. The overall 1-, 3-, 5-year survival rates, and median survival time of BDC patients were 87.9%, 48.4%, 38.4 %, and 33.7 months, respectively. The overall 1-, 3-, 5-year survival rates, and median survival time of CCC patients were 69.5%, 35.8%, 24.5 %, and 22.7 months, respectively. There was a significant difference in the overall survival between BDC and CCC patients (P = .0333).

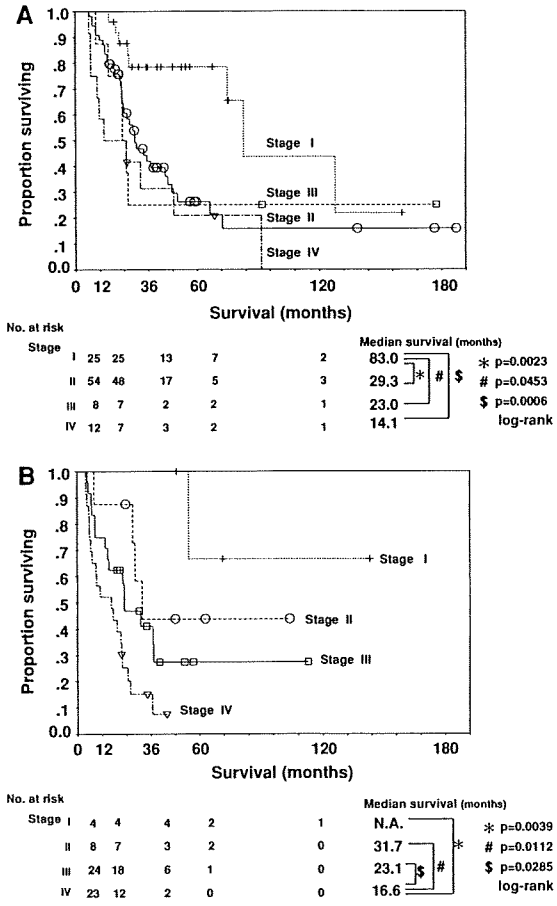

FIG. 4 Survival curves. (A) BDC patients by UICC pathological stage. Significant differences were noted between stages I and II (P = .0023), stages I and III (P = .0453), and stages I and IV (P = .0006). (B) The survival curves of CCC patients by UICC pathological stage. Significant differences were noted between stages I and IV (P = .0039), stages II and IV (P = .0112), and stages III and IV (P = .0285).

or distal (P = .028) bile duct margins were significantly more frequent in BDC patients. On the other hand, resected major portal vein invasion (P = .001) and moderate to severe venous invasion (P = .004) were significantly more frequent in CCC patients. There were no significant differences between BDC and CCC patients in the remaining six histopathological variables.

The 9 clinical and 11 histopathological risk factors possibly related to survival in BDC patients were analyzed by the log-rank test (Table 4). Male gender (P = .040), preoperative biliary drainage (P = .005), and an ICG R15 over 10% (P = .030) were significant clinical risk factors in BDC patients. Histologic differentiation (P = .010), depth of tumor invasion (P = .005), lymph node involvement (P < .001), resected major portal vein invasion (P = .009), venous invasion (P = .039), and nervous system invasion (P = .004) were significant histopathological risk factors in BDC patients.

The 8 clinical and 12 histopathological risk factors possibly related to survival in CCC patients were analyzed by the log-rank test (Table 5). Serum CA 19-9 (P = .006), CEA level (P = .002), and red blood cell transfusion requirement (P < .001) were significant clinical risk factors in CCC patients. Macroscopic tumor type (P = .004), resected major portal vein invasion (P = .011), T-category (P = .001), lymph node involvement (P = .016), lymphatic system invasion (P = .014), venous invasion (P = .017), nervous system invasion (P = .036), presence of intrahepatic daughter nodules (P = .003), and cancer-positive proximal bile duct margin (P = .003) were significant histopathological risk factors in CCC patients.

Multivariate analysis using the Cox proportional hazard model identified the curative resection with cancer-free margin (R0) (P = .024, odds ratio 1.862), the histologic type (well differentiated or papillary adenocarcinoma) (P = .011, odds ratio 2.135), and the absence of lymph node involvement (P < .001, odds ratio 3.314) as independent factors that contributed to

TABLE 3. *Histopathological variables*

Variable		BDC (n = 99)	CCC (n = 59)	P value
Histologic differentiation	Well, papillary	36 (36%)	12 (20%)	.034
T-category	1, 2	40 (40)	26 (44)	
Lymph node metastasis	Present	47 (47)	36 (61)	
Invasion of the resected major portal vein	Present	26 (26)	31 (53)	.001
Invasion of the lymphatic system	Absent or slight	62 (63)	29 (49)	
Invasion of the venous system	Absent or slight	65 (66)	25 (42)	.004
Invasion of the nervous system	Absent or slight	32 (32)	18 (31)	
Histological stage	I, II	79 (80)	12 (20)	<.001
Proximal ductal margin	Positive	29 (29)	9 (15)	.046
Distal ductal margin	Positive	17 (17)	3 (5)	.028
Dissected periductal margin	Positive	13 (13)	10 (17)	
R0 resection	Achieved	58 (59)	43 (73)	

Percentage are described in parentheses.

prolonged survival in BDC patients. On the other hand, the absence of intrahepatic daughter nodules (P < .001, odds ratio 2.318), preoperative serum CEA level of 2.9 ng/mL or less (P = .005, odds ratio 2.606), red blood cell transfusion requirement (P = .016, odds ratio 2.614), absence or slight degree of lymphatic system invasion (P < .001, odds ratio 4.577), and cancer-negative proximal bile duct margin (P = .003, odds ratio 7.398) were identified as independent factors that contributed to prolonged survival in CCC patients (Table 6).

DISCUSSION

The clinical impact of differentiating between BDC and CCC has not been clarified. In this setting, our present study is the first large, single-center series that has addressed the prognostic factors for BDC and CCC separately. Nakeeb et al.[21] evaluated the surgical outcome of cholangiocarcinoma divided into three categories: intrahepatic, perihilar, and distal cholang iocarcinoma. Their classification appears to be reasonable with respect to the choice of surgical procedure: hepatectomy for intrahepatic cholangiocarcinoma, hepatobiliary resection for perihilar cholangiocarcinoma, and the Whipple procedure for distal cholangiocarcinoma. Although perihilar cholangiocarcinoma can be divided into BDC and CCC based on the anatomical origin of the tumor, a substantial number of reports have described the surgical outcome of hilar cholangiocarcinoma, which have likely included CCC patients. We previously reported the safety and short-term outcome of major hepatobiliary resection for perihilar cholangiocarcinoma.[14] In the present study, we performed a prognostic analysis of perihilar cholangiocarcinoma patients treated with major hepatobiliary resection to delineate the characteristics of long-term survivors and to assess the impact of differentiating between BDC and CCC.

The overall survival of BDC patients was significantly better than that of CCC patients (Fig. 1, P = .033). This difference is potentially caused by a different distribution of the pathological stages in this study; CCC patients had a higher proportion of stage III or IV disease (74.6%) than BDC patients (25.3%, P < .001). In fact, there was no significant difference in the overall survival between BDC and CCC patients with the same stage. However, the validity of using the UICC staging system based on the TNM classification of extrahepatic bile duct cancer for BDC and liver cancer for CCC to compare the two groups might be questioned. Many of the possible risk factors that were analyzed are similar for both BDC and CCC, though on univariate analysis, only a few factors were significant predictors for both. On multivariate analysis, no significant independent prognostic factors were common for both BDC and CCC. Thus, BDC and CCC appear to show independent biological behaviors. Therefore, differentiating between BDC and CCC would have an impact on our ability to predict postoperative survival based on their independent prognostic factors.

BDC is typically associated with thickness or irregularity of the bile duct wall with or without involvement of adjacent liver parenchyma or portal structures. CCC is frequently associated with tumor bulk with or without invasion to Glisson's capsule on imaging studies; both BDC and CCC may show intraductal tumor extension.[17] Tumor bulk might be related to the higher CEA and CA19-9 levels seen in CCC than in BDC. Nevertheless, precise preoperative differentiation between BDC and CCC using various diagnostic imaging studies or clinical manifestation is

TABLE 4. *Possible clinical and pathological risk factors for survival in BDC (univariate analysis)*

Factors	No. of patients	Survival rate (%) 3-year	5-year	Median survival (months)	*P* value
Overall	99	48.4	38.4	33.7	
Age (median: 64 years)					
≤64	50	56.7	41.6	47.6	
>64	49	39.6	35.6	29.3	
Gender					.040
Male	69	43.6	31	30.1	
Female	30	58.8	53.9	72.7	
Biliary drainage					.005
Not performed	22	80.7	68.7	72.7	
Performed	77	38.5	28.8	26.7	
ICG R15 (normal range; ≤10%)					.030
≤10	62	54.4	45.8	47.6	
>10	37	38.2	26.4	26.5	
CA19-9 (median: 101 U/mL)					
≤101	50	48.3	38.0	33.7	
>101	49	48.7	39.6	35.5	
CEA (median: 2.5 ng/mL)					
≤2.5	53	41.5	27.8	26.7	
>2.5	46	55.8	51.9	66.4	
With PD					
Yes	10	41.1	41.1	32	
No	89	49.1	38.3	35.5	
With VR					
Yes	24	41.9	35.9	28.3	
No	75	50.4	39.1	37.2	
Red blood cell transfusion					
Performed	34	39.2	31.4	28.3	
Not performed	65	54	43	44.3	
Macroscopic type of the tumor					
Polypoid	9	63.5	47.6	45.5	
Nodular or infiltrative	90	47.0	37.7	32.0	
Histologic differentiation					
Well or papillary	36	60.0	56.3	83.0	.010
Others	63	42.0	25.9	26.5	
Depth of tumor invasion					
Mucosal, fibromuscle layer	8	100.0	100.0	N.A.	.005
Subserosal or more	91	43.8	33.7	29.3	
T category					
1, 2	40	59.6	59.6	83.0	
3, 4	59	41.1	25.0	29.1	
Lymph node involvement					<.001
Negative	52	67.3	58.4	75.2	
Positive	47	27.7	15.9	23.1	
Invasion of the resected portal vein					.009
Absent	73	55.7	51.1	66.4	
Present	26	30.8	13.2	23.5	
Invasion of the lymphatic system					
Absent or slight	62	54.4	46.6	45.5	
Moderate to marked	37	38.8	25.9	29.3	
Invasion of the venous system					.039
Absent or slight	65	57.4	46.0	47.6	
Moderate to marked	34	31.2	23.4	25.2	
Invasion of the nervous system					.004
Absent or slight	32	70.5	60.6	127.7	
Moderate to marked	67	38.2	28.3	26.0	
Proximal ductal margin					
Negative	70	54.1	41.1	45.5	
Positive	29	32.6	32.6	25.2	
R0 resection					
Achieved	58	57.2	44.4	45.5	
Not achieved	41	35.1	30.1	29.9	

ICGR15 indicates indocyanine green retention value at 15 minutes; CA19-9, carbohydrate antigen 19-9; CEA, carcinoembrionic antigen; PD, pancreatoduodenectomy; VR, vascular (hepatic artery, portal vein or inferior vena cava) resection and reconstruction; N.A., not available.

TABLE 5. *Possible clinical and pathological risk factors for survival in CCC (univariate analysis)*

Factors	No. of patients	Survival rate (%) 3-year	5-year	Median survival (months)	*P* value
Overall	59	35.8	24.5	22.7	
Age (median, 66 years)					
≤66	33	29.2	25.1	22.1	
>66	26	43.8	24.7	28.4	
Gender					
Male	37	37.6	28.2	21.5	
Female	22	34.1	21.9	24.7	
Biliary drainage					
Not performed	43	36.6	30.5	25.9	
Performed	16	33.3	11.1	17.5	
ICG R15 (normal range, ≤10%)					
≤10	43	36.7	30.1	32.1	
>10	16	34.7	13	25.9	
CA19-9 (median, 306 IU/mL)					.006
≤306	30	50.4	35	37	
>306	29	21.1	14.1	14.8	
CEA (median, 2.9 mg/dL)					.002
≤2.9	30	50.4	38.1	36.7	
>2.9	29	20.6	10.3	14.8	
With VR					
Yes	21	37.1	14.9	23.1	
No	38	34.9	27.9	22.1	
Red blood cell transfusion					<.001
Performed	26	11.5	3.9	17.5	
Not performed	33	58.4	45.9	54.9	
Macroscopic type of the tumor					.004
Mass-forming	44	25.8	12.3	17.5	
Periductal or intraductal	15	6.6	56.5	N.A.	
Serosal invasion					
Positive	7	21.4	0	7	
Negative	52	37.6	27.6	23.1	
Histologic differentiation					
Well or papillary	12	41.7	31.3	25.9	
Others	47	34.4	24.1	22.1	
Invasion of resected major portal vein					.011
Absent	29	49	38.5	31.7	
Present	30	22.7	N.A.	17.5	
T category					.001
1, 2	26	55.3	47.4	54.9	
3, 4	33	20.2	N.A.	17.5	
Lymph node involvement					.016
Negative	23	50.6	37.5	37.4	
Positive	36	26.8	17.9	17.5	
Invasion of the lymphatic system					.014
Absent or slight	29	52.6	33.8	36.7	
Moderate to marked	30	19	19	16.6	
Invasion of the venous system					.017
Absent or slight	25	45.4	40.3	31.1	
Moderate to marked	34	28.9	0	16.6	
Invasion of the nervous system					.036
Absent or slight	18	60.2	40.1	54.9	
Moderate to marked	41	25	16.7	21.5	
Intrahepatic daughter nodule					.003
Absent	42	44.4	31.3	31.1	
Present	17	14.1	7.1	11.1	
Proximal bile ductal margin					.003
Negative	50	40.5	29.8	27.2	
Positive	9	11.1	0	9.3	
R0 resection					
Achieved	42	40.6	29.5	27.2	
Not achieved	17	23.2	N.A.	21.5	

ICGR15 indicates indocyanine green retention value at 15 minutes; CA19-9, carbohydrate antigen 19-9; CEA, carcinoembrionic antigen; VR, vascular (hepatic artery, portal vein or inferior vena cava) resection and reconstruction; N.A., not available.

TABLE 6. *Risk factors associated with postoperative survival in patients with perihilar cholangiocarcinoma (Cox Multivariate Regression Analysis)*

Variable	β	SE	*P* value	Odds ratio	95% CI
BDC patients					
R0 resection	0.622	0.275	.024	1.862	1.085–3.194
Histologic differentiation (well, papillary versus others)	0.759	0.300	.011	2.135	1.186–3.844
Lymph node involvement	1.198	0.282	<.001	3.314	1.906–5.763
CCC patients					
Intrahepatic daughter nodule	0.841	0.239	<.001	2.318	1.450–3.705
Preoperative serum CEA level over 2.9 ng/mL	0.958	0.341	.005	2.606	1.337–5.080
Red blood cell transfusion requirement	0.961	0.399	.016	2.614	1.195–5.719
Invasion of the lymphatic system	1.521	0.423	<.001	4.577	1.997–10.494
Proximal bile duct margin	2.001	0.673	.003	7.398	1.976–27.688

$P < 0.1$ was set as the cut-off for variable elimination.

sometimes difficult. Actually, in 12 of 59 cases (20%) finally diagnosed as CCC, 17 of 99 cases (17%) finally diagnosed as BDC, it was not easy to discriminate between CCC and BDC by the review of pathologist. In our series, in approximately 20% of the cases it was not easy to discriminate between BDC and CCC. Hepatobiliary surgeons and pathologists should be aware of the differences between BDC and CCC. In addition, the examination of a greater number of cases and the use of immunohistological or genetic techniques may provide a better understanding of these two conditions.[22]

With respect to the surgical procedures, HPD[20] to secure the distal bile duct margin was done significantly more frequently in BDC patients. This difference implies that, among the BDC cases, there was extensive longitudinal extension along the extrahepatic bile duct, which may account for the significantly higher cancer-positive rates of the proximal and distal bile duct margins among the BDC patients. The rate of portal vein resection and reconstruction was comparable in the BDC and CCC patients. In our protocol, the decision to perform a right- or left-sided hepatectomy is made based on the predominant location of the tumor. When the tumor involved the right and left or proximal bile duct equally, right-sided hepatectomy was the first choice; this situation commonly occurs in BDC patients. Thus, a right-sided hepatectomy and prior PVE were performed significantly more frequently in BDC patients.

The bile duct margins must be clear of cancer to achieve cure; many previous reports have suggested that the presence of clear margins is an independent prognostic factor,[3–6,10–13,21] which is consistent with the results seen in our CCC patients. In BDC patients, the proportion of positive proximal bile duct margin was 29%, and R0 resection was achieved in 59%. This figure is lower than the 70% or greater cancer-negative surgical margins previously reported in large series.[3,5,7,9,12,13,23] The relatively high rate of cancer-positive surgical margins in the present study may be attributed to the strict diagnostic criteria used for bile duct margins or due to institutional differences in the diagnostic criteria. Nevertheless, the overall 5-year survival rate of 38.4% for BDC patients in our series is at least comparable to previous reports.[3,5,7,9,12,13,23] Further research is needed to clarify the diagnostic accuracy of bile duct margins[24,25] and the impact of positive bile duct margins on survival.[26,27]

Although the rates of red blood cell (RBC) transfusion requirement in both CCC and BDC group were comparable, CCC patients who underwent RBC transfusion showed a significantly shorter survival than those who did not undergo transfusion. On the other hand, there was no significant survival difference in BDC patients in terms of RBC transfusion. In patients with hepatocellular carcinoma (HCC), Yamamoto et al.[28] pointed out that perioperative RBC transfusion promotes tumor recurrence after hepatectomy. This may support that CCC, classified into primary liver tumor as same as HCC, potentially has a different character from BDC classified into bile duct tumor.

In summary, the overall survival of BDC patients was significantly better than that of CCC patients. On univariate analysis, only a few of the statistically significant clinicopathological factors were the same in the two groups; on multivariate analysis, there were no common significant predictive factors. Thus, BDC and CCC appear to show different biological behaviors. Differentiating between these two conditions would have an impact on the ability to predict postoperative survival.

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REFERENCES

1. Nimura Y, Hayakawa N, Kamiya J, Kondo S, Shionoya S. Hepatic segmentectomy with caudate lobe resection for bile duct carcinoma of the hepatic hilus. *World J Surg* 1990; 14:535–43.
2. Washburn WK, Lewis WD, Jenkins RL. Aggressive surgical resection for cholangiocarcinoma. *Arch Surg* 1995; 130:270–6.
3. Klempnauer J, Ridder GJ, von Wasielewski R, Werner M, Weiman A, Pichlmayr R. Resectional surgery of hilar cholangiocarcinoma: a multivariate analysis of prognostic factors. *J Clin Oncol* 1997; 15:947–54.
4. Neuhaus P, Jonas S, Bechstein WO, et al. Extended resections for hilar cholangiocarcinoma. *Ann Surg* 1999; 230:808–18.
5. Miyazaki M, Ito H, Nakagawa K, et al. Parenchyma-preserving hepatectomy in the surgical treatment of hilar cholangiocarcinoma. *J Am Coll Surg* 1999; 189:575–83.
6. Kosuge T, Yamamoto J, Shimada K, Yamasaki S, Makuuchi M. Improved surgical results for hilar cholangiocarcinoma with procedures including major hepatic resection. *Ann Surg* 1999; 230:663–71.
7. Nimura Y, Kamiya J, Kondo S, et al. Aggressive preoperative management and extended surgery for hilar cholangiocarcinoma: Nagoya experience. *J Hepatobiliary Pancreat Surg* 2000; 7:155–62.
8. Gerhards MF, van Gulik TM, de Wit LT, Obertop H, Gouma DJ. Evaluation of morbidity and mortality after resection for hilar cholangiocarcinoma: a single center experience. *Surgery* 2000; 127:395–404.
9. Lee SG, Lee YJ, Park KM, Hwang S, Min PC. One hundred and eleven liver resections for hilar bile duct cancer. *J Hepatobiliary Pancreat Surg* 2000; 7:135–41.
10. Seyama Y, Kubota K, Sano K, et al. Long-term outcome of extended hemihepatectomy for hilar bile duct cancer with no mortality and high survival rate. *Ann Surg* 2003; 238:73–83.
11. Kawasaki S, Imamura H, Kobayashi A, Noike T, Miwa S, Miyagawa S. Results of surgical resection for patients with hilar bile duct cancer: application of extended hepatectomy after biliary drainage and hemihepatic portal vein embolization. *Ann Surg* 2003; 238:84–92.
12. Hemming AW, Reed AI, Fujita S, Foley DP, Howard RJ. Surgical management of hilar cholangiocarcinoma. *Ann Surg* 2005; 241:693–702.
13. Jarnagin WR, Bowne W, Klimstra DS, et al. Papillary phenotype confers improved survival after resection of hilar cholangiocarcinoma. *Ann Surg* 2005; 241:703–14.
14. Sano T, Shimada K, Sakamoto Y, Yamamoto J, Yamasaki S, Kosuge T. One hundred two consecutive hepatobiliary resection for perihilar cholangiocarcinoma with zero mortality. *Ann Surg* 2006; 244:240–7.
15. Makuuchi M, Thai BL, Takayasu K, et al. Preoperative portal embolization to increase safety of major hepatectomy for hilar bile duct carcinoma: a preliminary report. *Surgery* 1990; 107:521–7.
16. Nagino M, Kamiya J, Nishio H, Ebata T, Arai T, Nimura Y. Two hundred forty consecutive portal vein embolizations before extended hepatectomy for biliary cancer: surgical outcome and long-term follow-up. *Ann Surg* 2006; 243:364–72.
17. Sano T, Kamiya J, Nagino M, et al. Macroscopic classification and preoperative diagnosis of intrahepatic cholangiocarcinoma in Japan. *J Hepatobiliary Pancreat Surg* 1999; 6:101–7.
18. In: Sobin LH, Wittekind C (eds). International Union Against Cancer (UICC): TNM Classification of Malignant Tumors 6th ed. New York: Wiley-Liss; 2002.
19. Cox D, Oakes D. Analysis of Survival Date London: Chapman and Hall; 1983.
20. Nimura Y, Hayakawa N, Kamiya J, et al. Hepatopancreatoduodenectomy for advanced carcinoma of the biliary tract. *Hepatogastroenterolog* 1991; 38:170–5.
21. Nakeeb A, Pitt HA, Sohn TA, et al. Cholangiocarcinoma: A spectrum of intrahepatic, perihilar, and distal tumors. *Ann Surg* 1996; 224:463–73.
22. Jarnagin WR, Klimstra DS, Hezel M, et al. Differential cell cycle-regulatory protein expression in biliary tract adenocarcinoma: correlation with anatomic site, pathologic variables, and clinical outcome. *J Clin Oncol* 2006; 24:1152–60.
23. Kondo S, Hirano S, Ambo Y, et al. Forty consecutive resections of hilar cholangiocarcinoma with no postoperative mortality and no positive ductal margins: results of a prospective study. *Ann Surg* 2004; 240:95–101.
24. Sakamoto E, Nimura Y, Hayakawa N, et al. The pattern of infiltration at the proximal border of hilar bile duct carcinoma: a histologic analysis of 62 resected cases. *Ann Surg* 1998; 227:405–11.
25. Ebata T, Watanabe H, Ajioka Y, Oda K, Nimura Y. Pathological appraisal of lines of resection for bile duct carcinoma. *Br J Surg* 2002; 89:1260–7.
26. Wakai T, Shirai Y, Moroda T, Yokoyama N, Hatakeyama K. Impact of ductal resection margin status on long-term survival in patients undergoing resection for extrahepatic cholangiocarcinoma. *Cancer* 2004; 103:1210–6.
27. Sakamoto Y, Kosuge T, Shimada K, et al. Prognostic factors of surgical resection in middle and distal bile duct cancer: an analysis of 55 patients concerning the significance of ductal and excisional margins. *Surgery* 2005; 137:396–402.
28. Yamamoto J, Kosuge T, Takayama T, et al. Perioperative blood transfusion promotes recurrence of hepatocellular carcinoma after hepatectomy. *Surgery* 1994; 115:303–9.

Expression and Gene Amplification of Actinin-4 in Invasive Ductal Carcinoma of the Pancreas

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Abstract

Purpose: An invasive growth pattern is one of the hallmarks of pancreatic ductal carcinoma. Actinin-4 is an actin-binding protein associated with enhanced cell motility, invasive growth, and lymph node metastasis. Actinin-4 might play an important role in the development and progression of pancreatic cancer.

Experimental Design: The expression of actinin-4 was examined immunohistochemically in 173 cases of invasive pancreatic ductal carcinoma. The copy number of the actinin-4 (*ACTN4*) gene was calculated by fluorescence *in situ* hybridization. The expression of actinin-4 was stably knocked down by short hairpin RNA, and tumorigenicity was evaluated by orthotopic implantation into mice with severe combined immunodeficiency.

Results: The expression level of actinin-4 was increased in 109 (63.0%) of 173 cases of pancreatic cancer. Kaplan-Meier survival curves revealed that patients with increased expression of actinin-4 had a significantly poorer outcome ($P = 0.00001$, log-rank test). Multivariate analysis by the Cox proportional hazard model showed that high expression of actinin-4 was the most significant independent negative predictor of survival (hazard ratio, 2.33; $P = 0.000009$). Amplification (defined as more than four copies per interphase nucleus) of the *ACTN4* gene was detected in 11 (37.9%) of 29 cases showing increased expression of actinin-4. Knockdown of actinin-4 expression inhibited the destructive growth of cancer cells in the pancreatic parenchyma.

Conclusion: Recurrent amplification of chromosome 19q13.1-2 has been reported in pancreatic cancer, but the exact target gene has not been identified. Actinin-4 contributes to the invasive growth of pancreatic ductal carcinoma, and *ACTN4* is one of the candidate oncogenes in this chromosome locus.

Invasive ductal carcinoma of the pancreas is one of the most aggressive forms of human malignancy, with a 5-year survival rate of <5% to 10% and a median survival of <6 months (1, 2). As a result, pancreatic cancer is the fourth leading cause of cancer death in the United States, and is the fifth in Japan (3). Massive local invasion to adjacent organs and/or metastasis to regional lymph nodes and distal organs are detected in the majority of patients at the time of diagnosis. To improve the prognosis of patients with pancreatic cancer, it will be necessary to elucidate the molecular mechanisms causing invasion and metastasis.

We have identified an actin-binding protein, actinin-4, as a biomarker of cancer invasion and metastasis (4). The expression of actinin-4 was closely associated with the invasive phenotype of breast cancer and was a prognostic indicator in patients with this disease (4). A microarray analysis revealed that actinin-4 was a significant prognostic indicator in patients with non-small cell lung cancer (5). The expression level of actinin-4 protein was increased in the majority of cases of colorectal cancer, and the increase in expression was most significant in dedifferentiated cancer cells infiltrating at the invasive front (6). In mouse models, colorectal cancer cells expressing actinin-4 showed infiltrative growth and metastasized into regional lymph nodes (6).

Oncogenic activation of the K-ras (*KRAS*) gene occurs in >90% of pancreatic ductal carcinomas and is detected even in premalignant intraepithelial lesions (7, 8). Transgenic mice with a K-ras^{G12D} transgene develop hyperplasia of ductal epithelial cells (9), but the hyperplastic lesions infrequently

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