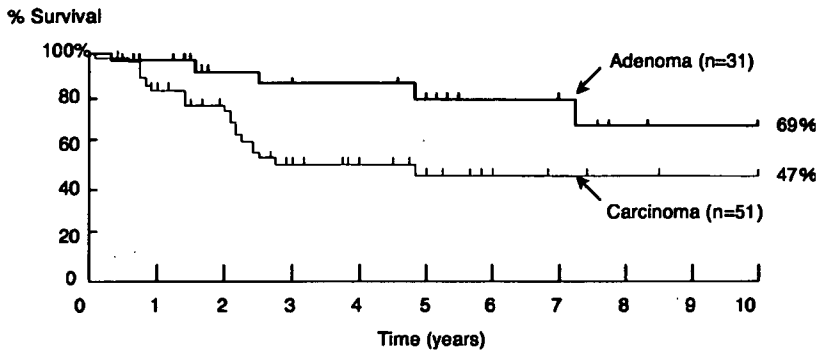

FIG. 1. Survival rates for patients with adenoma (n = 31) and carcinoma (n = 51).

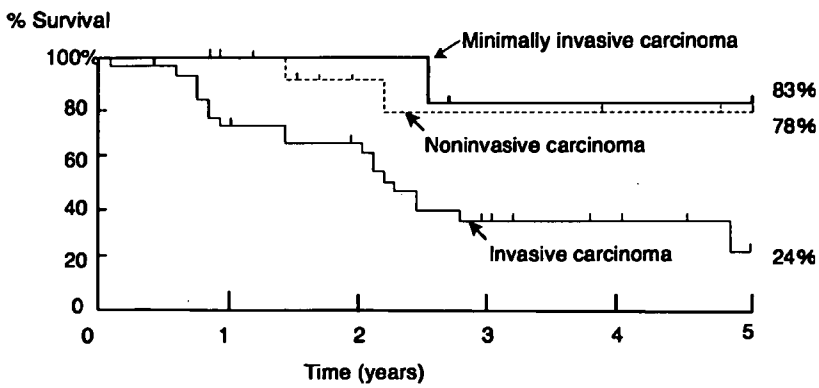

FIG. 2. Survival rates for patients with noninvasive carcinoma (n = 14), minimally invasive carcinoma (n = 6), and invasive carcinoma (n = 31).

TABLE 3. *Numbers of disease recurrence and death after surgical resection*

	Adenoma (n = 31)	Noninvasive (n = 14)	Minimally (n = 6)	Invasive (n = 31)
Numbers of death	5	2	1	19
Death due to recurrence	0	1	1	15
Death from other causes in disease-free patients	5	1	0	4
Numbers of recurrence	0	1	1	16
Recurrent site				
Locoregional	0	0	1	9
Peritoneum	0	1	1	8
Liver	0	0	0	6
Lung	0	0	0	2
Neck lymph node	0	0	0	1

Noninvasive, noninvasive carcinoma; Minimally, minimally invasive carcinoma; Invasive, invasive carcinoma.

as follows: locoregional (n = 10), peritoneum (n = 10), liver (n = 6), lung (n = 2), and neck lymph node (n = 1). Five disease-free patients with adenoma died of unrelated causes, such as lung cancer, gastric cancer, respiratory failure, renal failure, and ischemic heart disease. One disease-free patient with noninvasive carcinoma died of renal failure 17 months after the operation. Four disease-free patients with invasive adenocarcinoma died of unrelated causes, such as esophageal cancer, subarachnoid hemorrhage, pneumonia, and suicide. One patient with invasive carcinoma is alive with disease recurrence.

Clinicopathologic factors likely to influence survival after surgical resection are shown in Table 4. Among 13 factors, 11 were significantly associated with outcome in univariate analysis: age (≤65 vs >65), tumor size (≤4.0 vs >4.0 cm), maximum diameter of the main pancreatic duct (<5.0 vs ≥5.0mm), carcinoma, retroperitoneal invasion, intra-

TABLE 4. *Univariate analysis of potential predictors of overall survival after surgical resection*

Factors	No.	Survival (%) 1 year	3 years	5 years	*P* value
Age (years)					
≤65	41	95	84	79	.004
>65	41	82	45	40	
Gender					
Male	55	87	59	56	.26
Female	27	92	72	63	
Tumor size (cm)					
≤4.0	41	100	78	72	.033
>4.0	41	78	51	47	
Diameter of the MPD (mm)					
<5	24	95	89	81	.03
≥5	58	86	54	50	
Tumor type					
Main duct type	21	95	58	48	.74
Branch duct type	61	86	65	62	
Histology					
Adenoma	31	97	87	80	.017
Carcinoma	51	84	52	47	
Retroperitoneal invasion					
Negative	56	98	83	76	<.0001
Positive	26	68	26	26	
Intrapancreatic nerve invasion					
Negative	66	98	76	70	<.0001
Positive	16	50	16	0	
Venous invasion					
Negative	66	98	76	73	<.0001
Positive	16	47	10	0	
Lymph node metastases					
Negative	67	97	78	72	<.0001
Positive	15	53	10	0	
Margin status					
R0	73	90	73	67	<.0001
R1	9	78	0	0	
Serum CEA (ng/mL)					
<5.0	55	90	74	74	.0053
≥5.0	27	85	44	30	
Serum CA19-9 (U/mL)					
<37	52	98	78	71	.0007
≥37	30	73	35	35	

pancreatic nerve invasion, microscopic venous invasion, lymph node metastases, margin status, carcinoembryonic antigen (<5.0 vs ≥5.0 ng/mL), and carbohydrate antigen 19-9 (<37 vs ≥37 U/mL). Multivariate analysis using the Cox proportional hazards model was completed for 11 factors with a *P* value <.05 in univariate analysis. Multivariate analysis confirmed that lymph node metastasis ($P = .004$) and older age ($P = .015$) were significant and independent prognostic indicators after pancreatic resection for intraductal papillary mucinous neoplasms of the pancreas (Table 5). The overall 1-, 3-, and 5-year survival rates for patients with node-positive intraductal papillary mucinous carcinoma ($n = 15$) were 53%, 10%, and 0%, respectively. The overall 1-, 3-, and 5-year survival rates for patients with node-negative intraductal papillary mucinous neoplasm ($n = 67$) were 97%, 78%, and 72%, respectively. There was a significant difference in survival between patients with node-positive intraductal papillary mucinous neoplasm and those with a node-negative neoplasm ($P < .0001$).

DISCUSSION

The clinicopathologic features and surgical outcome of intraductal papillary mucinous neoplasm have not been fully clarified because this neoplasm is uncommon compared with pancreatic ductal carcinoma. Intraductal papillary mucinous neoplasms are frequently classified as noninvasive neoplasms and invasive neoplasms.[9,13,16] An indolent character and favorable outcome for noninvasive neoplasm have been described.[13,16,18] Conversely, poor survival results have been reported for invasive intraductal papillary mucinous neoplasm. In this study, the 5-year survival rate for invasive intraductal papillary mucinous carcinoma was 24%. Wada et al. reported that survival curves were not different between cases with invasive intraductal papillary mucinous neoplasm cases versus cases with ductal adenocarcinoma matched by stage.[18] Maire et al. reported that the overall 5-year survival rate was higher in patients with malignant invasive intraductal papillary mucinous neoplasm than in those with pancreatic ductal carcinoma, but was similar in the subset of stage II/III tumors.[19] These results suggest that invasive intraductal papillary mucinous neoplasm has a similar survival outcome to pancreatic ductal carcinoma. However, other authors reported that patients with invasive intraductal papillary mucinous neoplasm had a better outcome compared with those with pancreatic ductal carcinoma.[20–22] Salvia et al. reported that invasive intraductal papillary mucinous neoplasm had markedly favorable survival (60% at 5 years)[20]. Shimada et al. reported that the 5-year survival rate of invasive intraductal papillary mucinous neoplasm was significantly higher than that of common-type invasive ductal carcinoma (42% vs 20%).[21] Thus, further studies are needed to clarify the long-term survival of invasive intraductal papillary mucinous neoplasms.

It is difficult to accurately predict the outcome after surgical resection for invasive intraductal papillary mucinous neoplasm. Margin status might be an influential prognostic factor in patients with invasive carcinoma. This study showed that seven of nine patients with a carcinoma-positive margin died of

TABLE 5. *Multivariate Cox regression analysis of prognostic factors after surgical resection*

Factors	Relative risk	(95% CI)	*P* value
Lymph node metastases	5.53	(1.70–18.0)	.004
Age (> 65 years)	3.28	(1.26–8.53)	.015
Tumor size (> 4.0 cm)	0.99	(0.32–3.00)	.99
Diameter of the MPD (≥5 mm)	1	(0.24–4.26)	1
Histology (carcinoma)	0.76	(0.19–3.00)	.7
Retroperitoneal invasion	1.49	(0.28–7.89)	.64
Intrapancreatic nerve invasion	1.6	(0.13–19.5)	.71
Venous invasion	1.09	(0.09–13.2)	.94
Margin status (R1)	2.17	(0.70–6.71)	.18
Serum CEA (≥5.0 ng/mL)	1.78	(0.60–5.24)	.3
Serum CA19-9 (≥37 U/mL)	1.32	(0.50–3.54)	.58

recurrent disease, with a median survival time of 24 months. Sohn et al. reported that the 2-year survival rate for patients with invasive intraductal papillary mucinous neoplasm was only 40% when the margin was positive.[22] Thus, curative resection with a negative margin for carcinoma is essential for treating invasive neoplasm. In contrast, only a few patients with a positive margin for adenoma developed recurrent disease. In this study, 15 patients (five with adenoma, one with noninvasive carcinoma, two with minimally invasive carcinoma, and seven with invasive carcinoma) had a positive margin for adenoma. Of these 15 patients, recurrence occurred in four with invasive carcinoma and one with minimally invasive carcinoma. However, no recurrence occurred in five patients with intraductal papillary mucinous adenoma with a positive margin for adenoma. Chari et al. also reported that noninvasive intraductal papillary mucinous neoplasm recurs infrequently after resection, and survival is excellent regardless of the degree of epithelial dysplasia in the tumor.[16] D'Angelica et al. reported that the presence of atypia or carcinoma in situ at the ductal resection margin was not associated with a poor outcome.[14] These results suggest that a positive margin for adenoma is not associated with disease recurrence.

However, recurrent disease frequently occurred in patients with invasive intraductal papillary mucinous neoplasm even after margin-free resection. Despite a histological negative margin for carcinoma, 52% of patients (16 of 31) with invasive intraductal papillary mucinous neoplasm developed recurrence in our series. Chari et al. reported that invasive intraductal papillary mucinous neoplasm recurred frequently even after complete curative resection.[16] Cuillerier et al. also reported frequent recurrence not only after partial pancreatectomy with involved margins, but also after partial pancreatectomy with disease-free margins in patients with invasive neoplasm.[13] Identification of clinical and pathologic factors influencing survival is useful for the treatment of invasive neoplasms. Previous studies have demonstrated that tumor-associated biological factors, such as lymph node status, vascular invasion, and tumor size, are important in evaluating postoperative prognosis for invasive intraductal papillary mucinous neoplasm.[14,23] This study showed that nodal involvement was the independent predictor of poor survival in patients with intraductal papillary mucinous neoplasm. In this study, the overall 1-, 3-, and 5-year survival rates for patients with nodal involvement (n = 15) were 53%, 9%, and 0%, respectively, and node-positive patients had a significantly worse outcome than node-negative patients. Thus, pancreatectomy with lymph node dissection did not appear to improve survival in node-positive patients with an invasive neoplasm. Of node-positive invasive neoplasms, 93% had retroperitoneal invasion, 80% had intrapancreatic nerve invasion, 73% had microscopic venous invasion, and 33% had a positive margin for carcinoma. These results suggest that node-positive invasive intraductal papillary mucinous neoplasms appear to be in an advanced stage. Thus, resection with lymphadenectomy could not lead to a reduction in the incidence of recurrent disease. Further studies of adjuvant therapy combined with surgical resection are needed to achieve better survival results in invasive intraductal papillary mucinous neoplasm.

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Detail Histologic Analysis of Nerve Plexus Invasion in Invasive Ductal Carcinoma of the Pancreas and Its Prognostic Impact

Shuichi Mitsunaga, MD, PhD,† Takahiro Hasebe, MD,‡ Taira Kinoshita, MD,‡ Masaru Konishi, MD,‡ Shinichiro Takahashi, MD,‡ Naoto Gotohda, MD,‡ Toshio Nakagohri, MD,‡ and Atsushi Ochiai, MD**

Abstract: Nerve plexus invasion is regarded as one of the most important prognostic factors in invasive ductal carcinoma (IDC) of the pancreas, though nerve plexus invasion has not been evaluated in terms of prognostic impact on the basis of detailed histologic investigation. The purpose of this study was to precisely examine morphologic characteristics of nerve plexus invasion and analyze its prognostic predictive power compared with the well-known prognostic parameters of pancreatic IDCs. The outcome and histologic features of 75 patients with pancreatic IDC in the pancreas head were investigated, and 422 lesions of nerve plexus invasion were evaluated. Tumor cells invading nerve plexus showed a duct-forming differentiated feature and predominantly existed in the perineurium and perineural space. Multivariate analyses revealed that the important prognostic factors, in addition to invasive tumor size and tumor necrosis, were at long distances from nerve plexus invasion to pancreatic capsule and perineural invasion in nerve plexus invasion.

Key Words: nerve plexus invasion, pancreatic cancer, perineural space, nerve bundle, perineurium, neural invasion

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Pancreatic nerves converge outside pancreatic parenchyma and form a pancreatic nerve plexus.[25] The pancreatic nerve plexus was classified into the plexus pancreaticus capitalis, branching to pancreas head,[25–27] and the splenic plexus, branching to pancreas body and tail.[1,14] Pancreatic invasive ductal carcinoma (IDC) shows continuous spreading via neural routes.[16] Nerve invasion in pancreatic IDC was classified into intrapancreatic nerve invasion and pancreatic nerve plexus invasion (plx-inv), according to the position on pancreatic neural route.[10] Previous studies reported that intrapancreatic nerve invasion and plx-inv were observed in 90% to 100% and 69% to 81% of pancreatic IDC, and they emphasized the prognostic impact of plx-inv.[17,18] In our previous study,[15] plx-inv was found in 35% of IDCs of the pancreas and was the important prognostic factor, whereas intrapancreatic neural invasion, which was observed in approximately 100%, was not a prognostic factor. We hypothesized that nerve invasion was a common invasion behavior in pancreatic IDC and the level of nerve invasion correlated with survival period. The size of the cancer gland at neural invasion and the diameter of the invaded nerve reportedly had a prognostic impact on prostate cancer[13] and in oral squamous cell cancer.[4] Despite the high frequency of plx-inv in pancreatic IDCs, there are no systematic clinicopathologic studies investigating the detail histologic analysis and its prognostic impact of plx-inv. To identify a useful prognostic factor showing the malignant degree of nerve invasion, we planned to investigate the morphologic details of plx-inv.

The purpose of this study was to examine the histologic characteristics of plx-inv in IDC of the pancreas head in detail and further evaluate the prognostic value in patients with pancreatic IDC.

MATERIALS AND METHODS

Patients

Between September 1992 and January 2004, 75 patients who received a curative pancreaticoduodenectomy at our institution with a pathologic diagnose of pancreatic IDC were investigated. The median patient age was 65 years, where 32 patients were women. None of the patients received neo-adjuvant therapy before their initial operation. Regional lymph node dissection was performed in all patients, and portal vein resections were done in 34 patients. None of the 75 patients received adjuvant treatment.

From the *Pathology Division, Research Center for Innovative Oncology; ‡Surgical Pathology Section, Clinical Laboratory Division; and †Department of Upper Abdominal Surgery, National Cancer Center Hospital East, Kashiwa, Japan.

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Reprints: Atsushi Ochiai, MD, Pathology Division, Research Center for Innovative Oncology, National Cancer Center Hospital East, Kashiwanoha 6-5-1, Kashiwa, Chiba 277-8577, Japan (e-mail: aochiai@east.ncc.go.jp).

Histologic Examination

The resected specimens were fixed in 10% formalin at room temperature, and the size and gross appearance of the tumors were recorded. The specimens obtained through pancreaticoduodenectomy were sectioned along the long axis of the plexus pancreaticus capitalis[10,25–27] to precisely measure the distance of plx-inv (Figs. 1A–E). The entire tumor was sectioned at intervals of 0.5 to 0.7 cm, and all the tumor-containing sections were routinely processed and embedded in paraffin histologic examination. Serial sections (3 μm) of each tumor were cut. One section was stained with hematoxylin and eosin, and then examined pathologically to confirm the diagnosis.

One investigator (S.M.) assessed all the histologic parameters in this study, and another author (T.H.) identified the histologic characteristics of IDC to confirm the tumor cell characteristics assessed by S.M. Whenever a discrepancy occurred, both investigators reexamined the slides to reach a consensus.

Definition of plx-inv Evaluation

Criteria of plx-inv in this study were defined as follows: (A) nerve invasion at the plexus pancreaticus capitalis and (B) nerve invasion with isolation from main tumor and pancreatic tissue. Although perineural invasion was regarded as morphologic characteristics of nerve invasion in pancreatic IDC, intraneural IDC reportedly invaded another neural component, the nerve bundle.[9] The pancreatic nerve consisted of a nerve bundle, perineural space, perineurium, and epineurium.[2,5,12,21] In this study, the following 3 structures were evaluated: (1) nerve bundle where myelinated and unmyelinated nerve fibers consisting of an axon, Schwann cell, and endoneurium; (2) perineurium, which was one or several layers forming continuous epithelioid sheets consisting of spindle-shaped and flattened cells; and (3) perineural space, the internal space between nerve bundle and perineurium (Figs. 2A, B). On the basis of the above nerve structure definitions, IDC position in invaded nerves was classified into the following 3 categories: (1) nerve bundle invasion, and cancer cells clearly invading or irregularly compressed nerve bundles (Figs. 2C, D); (2) perineural space invasion, cancer cells existed in perineural space, and not showing any evidence of nerve bundle invasion (Figs. 2E, F); and (3) neurium invasion, cancer cells existed outer from perineural space, and attached to perineurium (Figs. 2G, H).

Parameters in the plx-inv Findings

Eight histopathologic parameters were evaluated in each of plx-inv, and are summarized in Table 1. The intraneural sites of IDCs in invaded nerves were morphologically evaluated as (1) predominant site, (2) deepest site, and (3) number of invaded sites. According to WHO classification[8] (4) predominant differentiation of IDCs in plx-inv was evaluated. (5) Nerve thickness and (6) cancer thickness were measured at plx-inv. (7) Distance from plx-inv to main tumor and (8) to pancreatic capsule were measured from the midpoint of short axis of nerve bundle in a plx-inv to the nearest main tumor and to the nearest pancreatic capsule. Pancreatic capsule was defined as the dense fibrotic layer outside the pancreas (Fig. 1D)

Parameters of plx-inv in Survival Analysis

The 9 parameters of plx-inv in each patient used for survival analysis were summarized in Table 1. (1) Predominant mode per patient, (2) deepest mode per patient, and (3) number of invaded mode per patient were evaluated as the most frequently observed predominant site of IDCs, deepest site of IDCs, and number of invaded sites of plx-inv in a patient. (4) Predominant differentiation per patient was defined as the most frequently observed differentiation of IDC of plx-inv in a patient. To analyze nerve thickness, cancer thickness, distance to main tumor and distance to pancreatic capsule in a patient, the actual values were evaluated based on amount beyond median value in 50% or more of plx-invs. Subsequently, the following 4 factors were categorized into low (< median) and high (≥ median, which was calculated on the data of 422 plx-invs): (5) nerve thickness per patient, (6) cancer thickness per patient, (7) distance to main tumor per patient, and (8) distance to pancreatic capsule per patient.

Clinicopathologic Parameters

Our previous report[15] precisely described the methods of the evaluation for the histologic parameters in this study. Fourteen histologic parameters were evaluated in this study according to WHO,[8] UICC,[22] and the Japan Pancreas Society[10] as follows: (a) invasive tumor size, (b) predominant differentiation, (c) least differentiation, (d) retroperitoneal invasion, (e) lymph vessel invasion, (f) blood vessel invasion, (g) intrapancreatic neural invasion, (h) UICC pT, (i) UICC pN, (j) UICC pStage, (k) tumor necrosis,[15,19] (l) fibrotic focus,[15,24] (m) portal vein invasion, and (n) portal vein resection.

Outcome

Seventy-five patients were followed for survival, and the follow-up period was calculated from the date of surgery until November 29, 2004. The median follow-up period was 1755 days (95% CI: 1566–2213). Overall, 62 patients died of their disease within the follow-up period.

Statistical Analysis

In a factor having 3 or more categories, for example predominant mode per patient, significant difference of survival between each categorical group was examined by Cox regression hazard model[7] between nerve bundle invasion and perineural space invasion, and between perineural space invasion and neurium invasion. These parameters were classified into 2 groups according to the most prognostic cut off that showed the most significant impact on survival in univariate analyses (data not shown). The reference arm in univariate analysis for plx-inv was the group without plx-inv. The cut off of actual data was determined as median value.

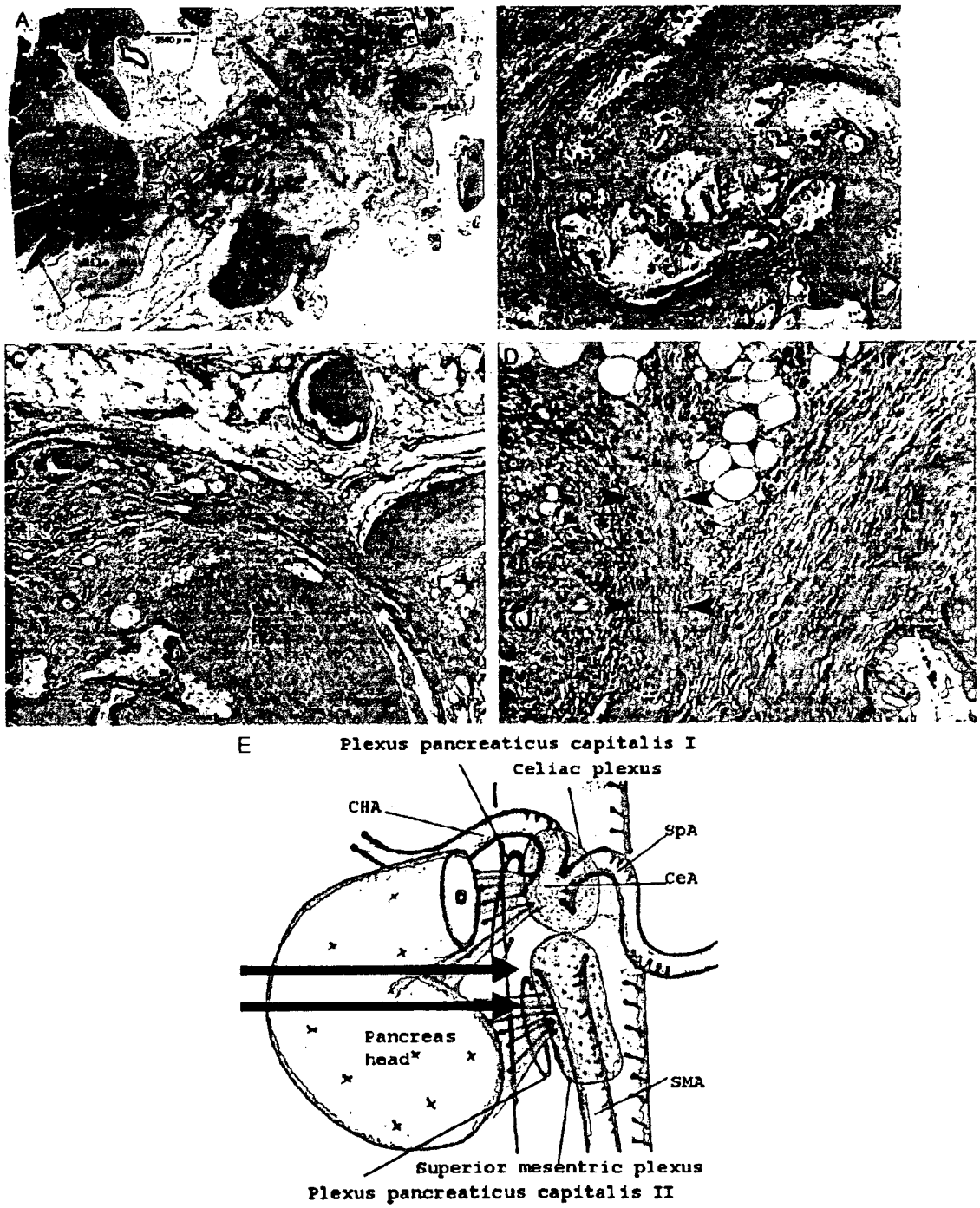

FIGURE 1. Shown is the demonstrable plexus invasion, a distance of 2500 μm from the pancreatic capsule, and the schema of nerve plexus network in the pancreas head. A Massive plexus invasion continuously existed in the plexus pancreaticus capitalis II (the area surrounded by the bold dots). A, distance of 2500 μm from the pancreatic capsule is shown (broken line). The right side of the nerve plexus was in close approximation to the superior mesenteric ganglion (the digital processed image using an objective lens magnification of 5 ×). B, The left side of plexus invasion near the pancreas (at an objective lens magnification of 4 ×). C, The right side of plexus invasion near the superior mesenteric ganglion (at an objective lens magnification of 4 ×). D, The densest layer of pancreatic capsule (at an objective lens magnification of 4 ×). E, Pancreatic nerves in pancreas head converge on plexus pancreaticus capitalis I or II, and achieve celiac plexus or superior mesenteric plexus. The pancreas head is sectioned approximately along the long axis of plexus pancreaticus capitalis (bold arrow). Ao indicates abdominal aorta; CeA, celiac artery; CHA, common hepatic artery; SpA, splenic artery; SMA, superior mesenteric artery.

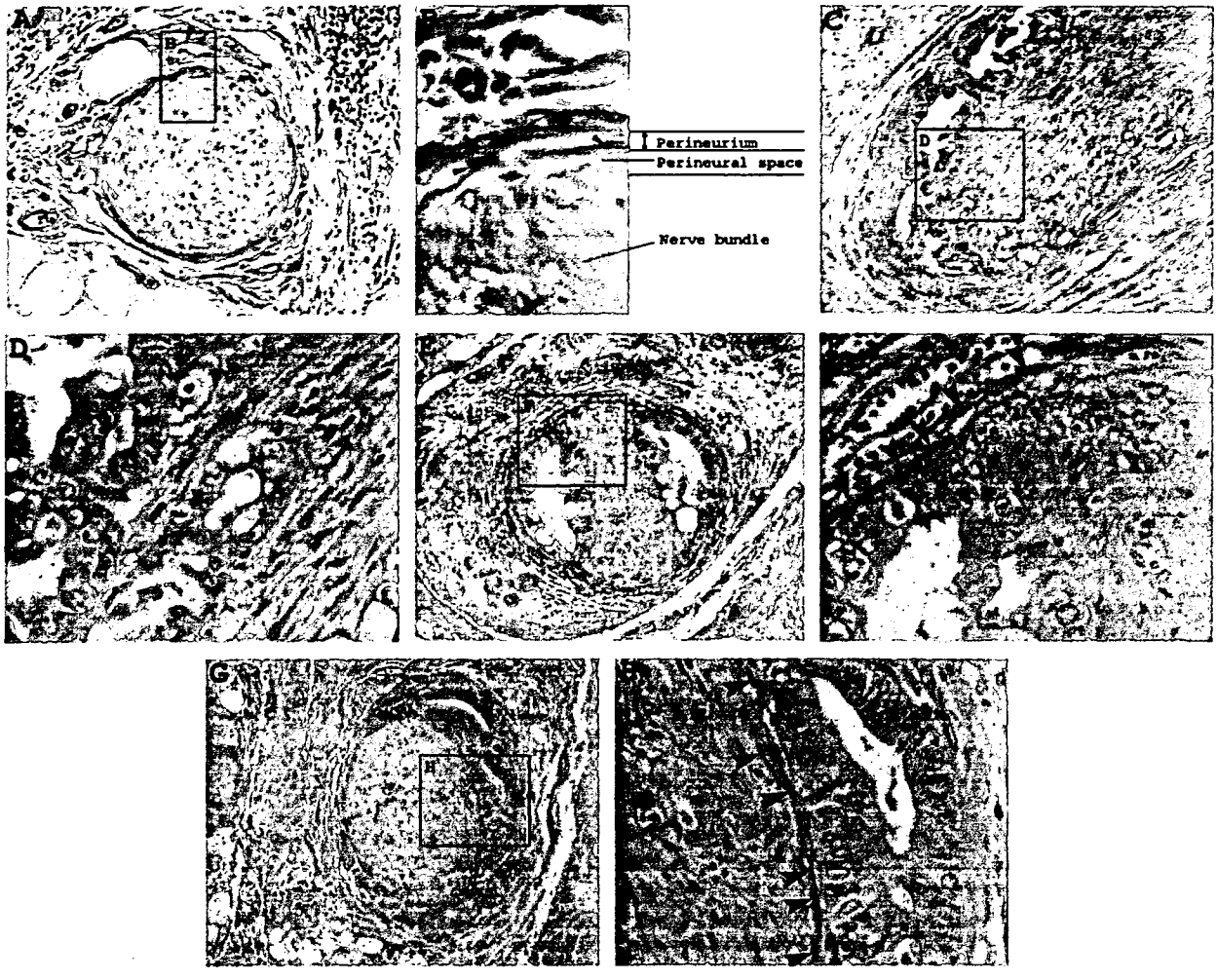

FIGURE 2. The structures of nerve (A, B) and the invaded neural structure classification (C–H). A, A nerve bundle was surrounded by the continuous layer like epithelioid sheets called the perineurium (at an objective lens magnification of 10 ×). B, Perineurium was one or several layers of continuous epithelioid sheets consisting of spindle shaped and flattened cells, called the perineurial cell (arrow head). The internal space between nerve bundle and perineurium was perineural space (at an objective lens magnification of 40 ×). C, Cancer cells clearly invaded the nerve bundle termed nerve bundle invasion. Cancer cells irregularly compressed the nerve bundle, and as seen on the left side, clearly invaded between nerve fibers (at an objective lens magnification of 10 ×). D, Invasive cancer cells clearly seen between nerve fibers. Cancer cells in nerve fibers tended to acquire low differentiation (at an objective lens magnification of 40 ×). E, Perineural space invasion seen as cancer cells in the perineural space. Almost all the cancer glands in perineural space invasion showed well-defined glandular shapes along the perineural space (at an objective lens magnification of 10 ×). F, Cancer glands invading the perineural space compressed the nerve bundle smoothly. The border between cancer cells and nerve bundles did not contain perineurial cells or perineurium. Perineurial cells (arrow head) consisting of perineurium are seen located at the outer aspect of the cancer gland (at an objective lens magnification of 40 ×). G, Neurium invasion was seen as cancer cells in the outer perineural space and attached to perineurium. Cancer glands in neurium invasion kept the well-defined glandular shape, and did not compress nerve bundle (at an objective lens magnification of 10 ×). H, Well-differentiated cancer cells in neurium invasion. Perineurial cells (arrow head) consisting of perineurium were between the nerve bundle and cancer gland (at an objective lens magnification of 40 ×).

The predictive parameters for survival in univariate analyses were analyzed in multivariate analyses using the Cox proportional hazard regression model. This initial multivariate analysis was performed and dealt only with plx-inv parameters or histologic parameters. The prognostic parameters in the initial multivariate analysis were analyzed together in multivariate analysis again to identify the strongest prognostic parameters. Overall survival curves were drawn using the Kaplan-Meier method.[11] Noncategorical data were compared using Mann-Whitney U test. All P values were 2-sided, and the significance level was set at $P < 0.05$. All analyses were performed using the Statview-J 5.0 package, Windows version (SAS, Inc, Cary, NC).

TABLE 1. The Explanation of Parameters to Evaluate the Morphologic Characteristic of Nerve Plx-Inv and Its Prognostic Impact in IDC of the Pancreas

No.	Parameters	Meanings	Variables
Parameters in the findings of plx-inv			
1	Predominant site	The predominant site of plx-inv	nb/pn/neu
2	Deepest site	The deepest site of plx-inv from outer of nerve	nb/pn/neu
3	Number of invaded sites	The numbers of sites of plx-inv	1/2/3
4	Predominant differentiation	Predominant differentiation of IDC in a plx-inv	wel/mod/por
5	Nerve thickness	Short axis of nerve bundle in a plx-inv	Actual (μm)
6	Cancer thickness	Short axis of IDC gland in a plx-inv	Actual (μm)
7	Distance to main tumor	The distance from plx-inv to the nearest main tumor	Actual (μm)
8	Distance to pancreatic capsule	The distance from plx-inv to the nearest pancreatic capsule	Actual (μm)
Parameters of plx-inv in survival analysis			
1	Predominant mode per patient	The most frequently observed predominant site of plx-inv in a patient	nb/pn/neu
2	Deepest mode per patient	The most frequently observed deepest site of plx-inv in a patient	nb/pn/neu
3	Number of invaded mode per patient	The most frequently observed number of sites of plx-inv in a patient	1/2/3
4	Predominant differentiation per patient	The most frequently observed predominant differentiation of IDC in a patient	Wel/mod/por
5	Nerve thickness per patient	Determination whether short axis of nerve bundle in 50% or more of plx-invs was beyond median value or not in a patient	Low/high
6	Cancer thickness per patient	Determination whether short axis of IDC gland in 50% or more of plx-invs was beyond median value or not in a patient	Low/high
7	Distance to main tumor per patient	Determination whether the distance from plx-inv to the nearest main tumor in 50% or more of plx-invs was beyond median value or not in a patient	Low/high
8	Distance to pancreatic capsule per patient	Determination whether the distance from plx-inv to the nearest pancreatic capsule in 50% or more of plx-invs was beyond median value or not in a patient	Low/high
9	Number of plx-inv	Determination whether the number of plx-inv was beyond median value or not in a patient	Low/high

mod indicates moderate differentiated; nb, nerve bundle invasion; neu, neurium invasion; pn, perineural invasion; por, poorly differentiated; wel, well differentiated.

RESULTS

Characteristics of Nerve Plx-Inv Foci

Plx-inv was found in 49 patients (65%). Of all the tumors from these 49 patients, 129 sections contained plx-inv (mean 2.7 ± 1.9 sections per patient). Total and median numbers of plx-inv were 422 and 6. Table 2 shows the morphologic characteristics of plx-inv. Predominant site of plx-inv was observed at perineural space invasion in 34%, at neurium invasion in 48%, and at nerve bundle invasion in 18%. The deepest site of plx-inv was detected evenly in the nerve structures (nerve bundle invasion 35%, perineural space invasion 27%, neurium invasion 38%). Eighty-six percent of IDCs in plx-inv maintained the ductal structure (well differentiation 58%, moderate differentiation 28%), and poorly differentiated IDC was found in 14%. Nerve thickness (thickness of invaded nerve) and the median cancer thickness in plx-inv were 125 and 100 μm, respectively. The median value of the distance from plx-inv to main tumor or pancreatic capsule was 2500 μm.

Survival Outcome

Median survival time in the group without plx-inv, with plx-inv, and all patients were 707, 371, and 400 days, respectively (overall survival curve is shown in Fig. 3A).

Univariate and Multivariate Analyses in Parameters of Nerve Plx-Inv and Orthodox Factors to Identify Important Prognostic Factors

Nine parameters dealing with plx-inv possessed the potential prognostic factors with univariate analysis

TABLE 2. Characteristics of Nerve Plexus Invasion in the Patients Who Received Macroscopic Curative Pancreaticoduodenectomy for IDC of the Pancreas

No.	Parameter	Value
1	Predominant site [plx n.(%)] nb/pn/neu	77(18)/143(34)/202(48)
2	Deepest site [plx n.(%)] nb/pn/neu	147(35)/114(27)/161(38)
3	Number of invaded sites [plx n.(%)] 1/2/3	208(49)/134(32)/80(19)
4	Predominant differentiation [plx n.(%)] well/mod/por	247(58)/119(28)/56(14)
5	Nerve thickness (μm) median (95%CI)	125 (125-150)
6	Cancer thickness (μm) median (95%CI)	100 (87.5-100)
7	Distance to main tumor (μm) median (95%CI)	2500 (2250-2625)
8	Distance to pancreatic capsule (μm) median (95%CI)	2500 (2200-2750)

CI indicates confidential interval; mod, moderately differentiated; nb, nerve bundle invasion; neu, neurium invasion; Plx n, the number of the invaded nerve plexus; pn, perineural space invasion; por, poorly differentiated; well, well differentiated.

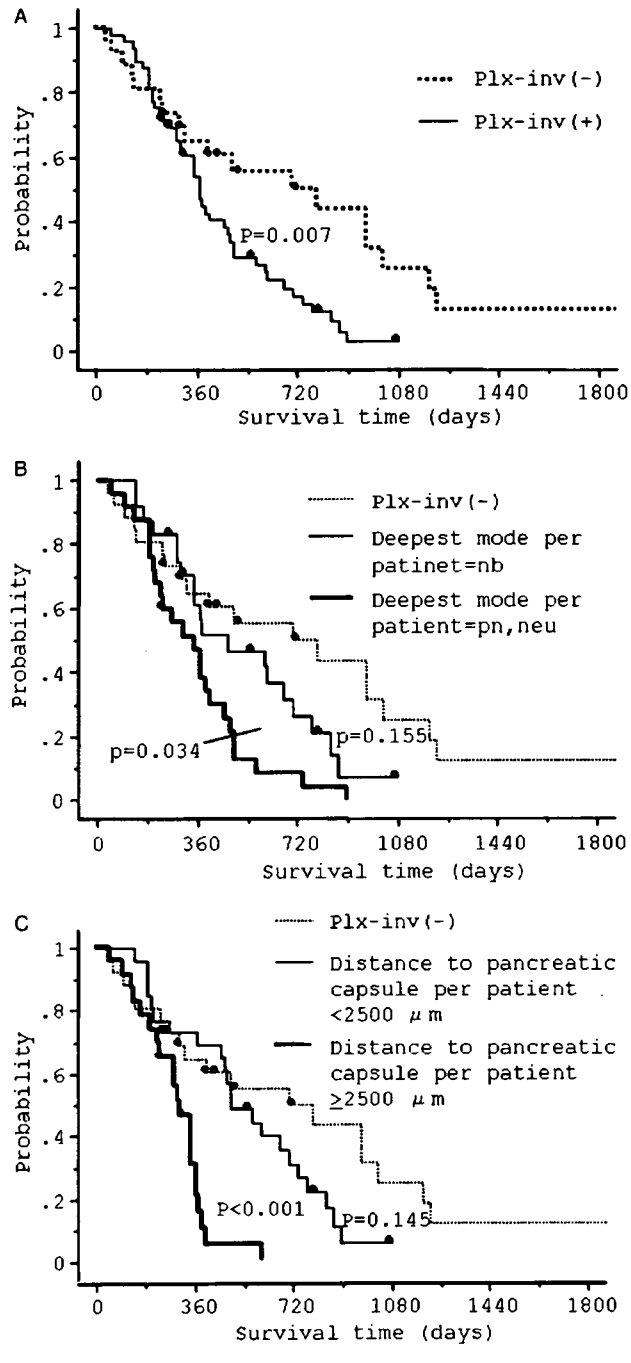

FIGURE 3. The overall survival curves according to the important factors of nerve plexus invasion (plx-inv) in all patients. A, Overall survival curves with or without plx-inv. The patients with nerve plx-inv showed a significantly poor prognosis. B, Survival curves according to deepest mode of plx-inv per patient. The developed manner of plx-inv without nerve bundle invasion significantly influenced poor prognosis. C, Survival curves according to distance from plx-inv to pancreatic capsule per patient. The patients in which plx-inv was predominantly 2500 μm or more away from pancreatic capsule showed a significantly poor prognosis. nb indicates nerve bundle invasion; pn, perineural space invasion; neu, neurium invasion.

(Table 3). Multivariate analysis-revealed independent prognostic factors among the parameters of plx-inv were perineural space invasion or neurium invasion in deepest mode per patient and distance to pancreatic capsule per patient (Table 3, and overall survival curves are shown in

TABLE 3. Univariate and Multivariate Analyses Using the Factors of Nerve Plexus Invasion in the Patients who Underwent Macroscopic Curative Pancreaticoduodenectomy for IDC of the Pancreas

No.	Parameter	n	Death (%)	HR	95% CI	P
Univariate analysis						
	Nerve plexus invasion					
	Absent	26	19 (73)	ref		ref
	Present	49	43 (88)	2.2	1.2-4.1	0.009
1	Predominant mode per patient					
	nb	7	5 (71)	1.5	0.5-4.1	0.469
	pn, neu*	42	38 (90)	2.4	1.3-4.5	0.005
2	Deepest mode per patient					
	Nb	24	19 (79)	1.7	0.9-3.4	0.126
	pn, neu*	25	24 (96)	3.2	1.6-6.3	< 0.001
3	Number of invaded mode per patient					
	< 3	21	21 (100)	2.2	1.1-4.4	0.021
	≥ 3*	28	22 (79)	2.3	1.2-4.4	0.018
4	Predominant differentiation per patient					
	Well	28	23 (82)	1.9	0.9-3.7	0.053
	Mod, por*	21	20 (95)	2.9	1.5-5.9	0.027
5	Nerve thickness per patient (μm)					
	< 125	17	16 (94)	1.8	0.9-3.7	0.103
	≥ 125*	32	27 (84)	2.6	1.4-5.0	0.004
6	Cancer thickness per patient (μm)					
	< 100*	25	23 (92)	2.6	1.3-5.1	0.005
	≥ 100	24	20 (83)	1.9	0.9-3.8	0.055
7	Distance to main tumor per patient (μm)					
	< 2500*	20	19 (95)	2.5	1.2-5.0	0.011
	≥ 2500	29	24 (83)	2.1	1.1-4.0	0.028
8	Distance to pancreatic capsule per patient (μm)					
	< 2500	26	22 (85)	1.6	0.8-3.2	0.149
	≥ 2500*	23	21 (91)	4.8	2.3-10.3	< 0.001
9	Numbers of plx-inv					
	< 6	22	20 (91)	2.1	1.1-4.2	0.030
	≥ 6*	27	23 (85)	2.3	1.2-4.5	0.012
Multivariate analysis						
2	Deepest mode per patient					
	pn, neu	25	24 (96)	2.1	1.2-3.7	< 0.001
8	Distance to pancreatic capsule per patient (μm)					
	≥ 2500	23	21 (91)	3.2	1.7-6.0	< 0.001

Univariate analysis was performed with cox regression hazard model referring to the patients without nerve plexus invasion (n = 26, deaths = 19). The factors with * at the end of a word were used for multivariate analysis in this table. Cox regression hazard model was used in multivariate analysis. Significant level was set at < 0.05.

CI, confidence interval; HR, hazard ratio; mod, moderately differentiated; nb, nerve bundle invasion; neu, neurium invasion; pn, perineural space invasion; por, poorly differentiated; well, well differentiated.

TABLE 4. Univariate and Multivariate Analyses Using the Factors of Main Tumor in the Patients Who Underwent Macroscopic Curative Pancreaticoduodenectomy for Invasive Ductal Carcinoma of the Pancreas

No.	Parameter	n	Deaths	HR	95% CI	P
Univariate analysis						
a	Invasive tumor size					
	≤3.0	38	27 (71)	ref		ref
	> 3.0*	37	35 (95)	2.7	1.6-4.6	< 0.001
b	Predominant differentiation					
	well, mod	65	52 (80)	ref		ref
	por	10	10 (100)	1.6	0.8-3.2	0.199
c	Lowest differentiation					
	well, mod	37	29 (78)	ref		ref
	por	38	33 (87)	1.0	0.6-1.7	0.907
d	Retroperitoneal invasion					
	0, 1	31	25 (81)	ref		ref
	2, 3	44	37 (84)	1.4	0.8-2.3	0.260
e	Lymph vessel invasion					
	0, 1	52	40 (77)	ref		ref
	2, 3*	23	22 (96)	1.8	1.0-3.0	0.038
f	Blood vessel invasion					
	0, 1	8	7 (88)	ref		ref
	2,3	67	55 (82)	1.9	0.9-4.2	0.111
g	Intrapancreatic neural invasion					
	0, 1	21	17 (81)	ref		ref
	2, 3	54	45 (83)	1.4	0.8-2.6	0.243
h	UICC pT					
	Pt3	71	58 (82)	ref		ref
	pT1, pT2	4	4 (100)	5.7	1.9-17.1	0.002
i	UICC pN					
	pN0	12	7 (58)	ref		ref
	pN1*	63	55 (87)	2.6	1.2-5.8	0.018
j	UICC pStage					
	IA, IB, IIA	15	10 (67)	ref		ref
	IIB, III, IV	60	52 (87)	1.8	0.9-3.6	0.095
k	Tumor necrosis					
	Absent	52	39 (75)	ref		ref
	Present*	23	23 (100)	2.2	1.3-3.7	0.004
l	Fibrotic focus					
	Absent	33	24 (73)	ref		ref
	Present	42	38 (90)	1.3	0.8-2.2	0.293
m	Portal vein invasion					
	Absent	57	48 (84)	ref		ref
	Present	18	14 (78)	1.1	0.6-1.9	0.863
n	Portal vein resection					
	Absent	41	35 (85)	ref		ref
	Present	34	27 (79)	0.7	0.4-1.2	0.152
Multivariate analysis						
a	Invasive tumor size					
	> 3.0	37	35 (95)	2.2	1.2-3.8	0.007
e	Lymph vessel invasion					
	2, 3	23	22 (96)	1.6	0.9-2.8	0.116
i	UICC pN					
	pN1	63	55 (87)	2.0	0.9-4.7	0.100
k	Tumor necrosis					
	Present	23	23 (100)	1.9	1.1-3.3	0.027

Univariate analysis was performed with cox regression hazard model. The factors with * at the end of a word were used for multivariate analysis in this table. Cox regression hazard model was used in multivariate analysis. Significant level was set at $P < 0.05$.

CI indicates confidence interval; HR, hazard ratio; mod, moderately differentiated; por, poorly differentiated; UICC, International union against cancer; well, well differentiated; 0, none; 1, slightly seen; 2, occasionally seen; 3, frequently seen.

TABLE 5. Multivariate Analysis Using the Important Factors of Nerve Plexus Invasion and Main Tumor in Patients Who Underwent Macroscopic Curative Pancreaticoduodenectomy for IDC of the Pancreas

Parameter	n	HR	95% CI	P
Distance to pancreatic capsule per patient				
≥ 2500 μm	23	2.8	1.5-5.3	0.001
Deepest mode per patient				
pn, neu	25	2.1	1.2-3.6	0.008
Invasive tumor size				
> 3.0 cm	48	2.3	1.3-3.9	0.004
Tumor necrosis				
Present	23	2.3	1.3-3.9	0.049

Cox regression hazard model was used in multivariate analysis. Significant level was set at $P < 0.05$.

CI indicates confidential interval; HR, hazard ratio; nb, nerve bundle invasion; neu, neurium invasion; pn, perineural space invasion.

Fig. 3B, C). With regard to histologic factors of main tumor, invasive tumor size and tumor necrosis possessed an independent predictive prognostic power (Table 4). Multivariate analysis of these four independent parameters showed their significant hazard ratio of death (Table 5). Invasive tumor size was recognized as the useful and powerful prognostic factor in oncology; however, the prognostic power of distance from plx-inv to pancreatic capsule exceeded that of invasive tumor size.

Distance to Pancreatic Capsule in the Groups Assigned by Deepest Site

To confirm the independence between the deepest invaded site of plx-inv and the distance from plx-inv to pancreatic capsule, 422 plx-inv were divided into nerve bundle invasion group or not, according to the deepest invaded site of plx-inv, and the distance from plx-inv to pancreatic capsule was compared between these 2 groups. These groups had similar values (nerve bundle invasion, n = 147, 2625 μm; perineural space invasion or neurium invasion, n = 275, 2250 μm) and were not significantly different ($P = 0.360$ in U test).

DISCUSSION

This study clearly demonstrates a long distance between nerve plx-inv and pancreatic capsule is very important in predictive prognosis of patients with pancreatic IDC. In addition, perineural space or neurium invasion as well as invasive tumor size and tumor necrosis are important prognostic factors. This is the first study to evaluate and quantify the morphologic detail and its prognostic impact in nerve invasion of pancreatic IDC.

Long distance (≥ 2500 μm) from plx-inv to pancreatic capsule is significantly associated with a shortened survival period. To discuss the relevance of the distance from plx-inv to pancreatic capsule, it is necessary to mention the relationship between pancreatic neural route, sectioning, and IDC nerve invasion. The nerve plexus of the pancreas head runs from the pancreas to celiac or

superior mesenteric plexus at approximately right angles from the abdominal aorta (Fig. 1A, E).[25–27] The resected specimen after a pancreaticoduodenectomy was sectioned at a right angle from the abdominal aorta.[10] Therefore, the sections are parallel to the nerve plexus route in the pancreas head. This directional alignment indicates the distance from plx-inv to pancreas approximates the extrapancreatic nerve length with invasion. As nerve invasion is continuous from the primary tumor,[20] this length is the developed distance of IDCs in the extrapancreatic nerve. On the basis of the above context, the developed distance of intraneural IDC is approximately equal to the distance from plx-inv to pancreatic capsule, and is considered to be an important prognostic factor. As this long distance of intraneural IDCs may correspond to the high capacity of tumor cells spreading in nerve, intraneural spreading ability of pancreatic IDC should be the focus of future studies. On the other hand, the distance to the main tumor is not a useful prognostic factor, and is considered insufficient when evaluating the developed distance of intraneural IDCs. Thus, the distance from the plx-inv should be assessed with respect to the pancreatic capsule and not the main tumor.

This study also demonstrates the significant prognostic effect of perineural space invasion or neurium invasion of tumor cells, but not nerve bundle invasion of the tumor cells. This result means that perineural invasion is an important prognostic factor. Perineural invasion is characteristic of nerve invasion in pancreatic IDC and its high prevalence is quantified in this study. The morphologic difference between nerve bundle invasion and perineural invasion is the disruption of nerve bundle owing to IDC invasion. According to Sunderland's[23] classification, which arranges peripheral nerve injuries in ascending order of severity from the first to the fifth degree corresponding to injury to myelin, axon, endoneurium, perineurium, and the entire nerve trunk, nerve bundle invasion (the third-degree injury) leads to more severe nerve damage than perineural invasion (the first to second-degree injury). Severe nerve damage results in poor nerve function,[23] and may change the interaction between IDC and nerve system. Anatomically, pancreatic nerve plexus is one of the pancreatic neural networks between pancreas and spine,[6] and connects each other through the nerve bundle. Nerve bundle invasion amputates this neural network, gives severe neural damage, and may lose tumor spreading acceleration via a neural network. On the other hand, perineural invasion leads to mild neural damage, is able to use functional neural network through the preservation of nerve bundle, and may result in local recurrence or other incidence.

Invasive tumor size and tumor necrosis were the important prognostic factors in our previous study,[15] and are reconfirmed in our current study. The distance from plx-inv to pancreatic capsule and perineural invasion shows the independent predictive prognostic power of invasive tumor size and tumor necrosis in multivariate analysis. Furthermore, there is no significant association between the 2 prognostic factors of plx-inv. Therefore, the prognostic value of the 2 plx-inv factors is validated in pancreatic IDC.

The determination of optimal section to evaluate plx-inv will be an important problem in the future. Careful tissue sectioning of pancreatic neural route is necessary considering the findings of this study. Anatomically, pancreatic neural route is elucidated in detail, and the determination of neural route sections may be useful when selecting optimal sections to evaluate plx-inv. The current data are the result of evaluations of entire tumor sections. Thus, the confirmation of the above hypothesis needs further study.

Although the mechanism of plx-inv is still unknown, the 2 prognostic parameters of plx-inv indicate that perineural development ability of tumor cell are worthy of further study of pancreatic IDC. An electron microscopic study reported that perineural pancreatic IDC attaches endoneurium and perineurium.[3] It is speculated that intraneural extracellular matrix is associated with perineural IDCs. Neural extracellular matrix might accelerate motility and/or proliferation of IDCs in the nerve, resulting in high ability of perineural development in pancreatic IDC. The ability of perineural development should be the focus for further study of pancreatic IDC.

In conclusion, 2 prognostic parameters in plx-inv for pancreatic IDC were revealed in this study: (1) the distance from plx-inv to pancreatic capsule was 2500 μm or more and (2) the invasive mode of tumor cells in nerve bundles. These results indicate that the long distance between intraneural IDCs and perineural invasion are important invasive predictors in pancreatic IDC. Pathologists should keep these in mind, along with invasive tumor size and tumor necrosis, in routine histologic examination of pancreatic IDCs.

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CASE REPORT

Adenocarcinoma of the minor duodenal papilla with intraepithelial spread to the pancreatic duct

Masatoshi Kajiwara · Satoshi Fujii · Shinichiro Takahashi · Masaru Konishi · Toshio Nakagohri · Naoto Gotohda · Taira Kinoshita

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Abstract It is extremely rare to encounter tumors arising exclusively in the minor duodenal papilla. We report a 60-year-old male patient with a polypoid type of adenocarcinoma of the minor papilla. Preoperative examinations, including computed tomography (CT) and magnetic resonance cholangiopancreatography (MRCP), suggested pancreas divisum and showed a series of stones in the dorsal pancreatic duct. The patient underwent subtotal stomach-preserving pancreaticoduodenectomy (SSpPD). On histology, an adenocarcinoma was located in the minor papilla, which was limited to the mucosa, without invasion of the duodenum, sphincter muscles of the minor papilla, or the underlying pancreas. The carcinoma cells, together with dysplastic and hyperplastic epithelium of the pancreatic duct, extended peripherally within the pancreatic duct. No cystic dilatation of the pancreatic duct was observed. The ventral pancreatic duct was short and narrow; there was evidence of chronic pancreatitis in the dorsal pancreas, whereas the ventral pancreas was almost normal, suggesting the existence of pancreas divisum. Although it is well known that adenocarcinoma of the duodenal papilla is sometimes accompanied by intraepithelial spread in the pancreatic duct, an adenocarcinoma arising in the minor papilla in this case with pancreas divisum was more extended than our thoughts.

Keywords Adenocarcinoma · Minor duodenal papilla · Intraepithelial spread · Pancreas divisum

M. Kajiwara · S. Takahashi (✉) · M. Konishi · T. Nakagohri · N. Gotohda · T. Kinoshita
Department of Hepatobiliary Pancreatic Surgery,
National Cancer Center Hospital East,
6-5-1 Kashiwanoha, Kashiwa,
Chiba 277-8577, Japan
e-mail: shtakaha@east.ncc.go.jp

S. Fujii
Pathology Division, Research Center for Innovative Oncology,
National Cancer Center Hospital East, Kashiwa,
Chiba, Japan

Introduction

Tumors of the minor duodenal papilla are rare. Most reported cases have been benign tumors, including carcinoid [12, 15, 19, 22, 23], somatostatinoma [3, 13, 20], adenoma [21], adenomyoma [4], and gangliocytic paraganglioma [11, 14]; only one case of adenocarcinoma has been previously reported [24]. Tumors in the minor papilla may be underestimated because of the difficulty in determining the origin of tumors that involve the papilla because the papilla may be overgrown even by small tumors, and the histological appearance of periampullary tumors is similar [1].

We present a case of primary adenocarcinoma of the minor duodenal papilla that exhibited wide intraepithelial spread in the pancreatic duct. Moreover, the existence of pancreas divisum was also suspected because of the distribution of pancreatitis on microscopic examination.

Clinical history

A 60-year-old male patient complained of transient abdominal pain for 2 months. His general practitioner performed gastroduodenoscopy and found a polypoid tumor of the duodenum. The patient was referred to the Department of Hepatobiliary Pancreatic Surgery at the National Cancer

Center Hospital East for further evaluation and treatment. The patient had no history of alcohol abuse.

Material and methods

The surgical specimen was fixed in 10% buffered formalin and submitted entirely for histology. The paraffin-embedded tissue were sectioned and stained with hematoxylin and eosin (H&E). Subsequently, tissue samples were stained immunohistochemically with the following monoclonal antibodies: cytokeratin (CK) 7 (1:100; Dako), CK20 (1:50; Dako), Muc-2 glycoprotein (1:100; Novocastra laboratories), and Muc-5AC glycoprotein (1:50; Novocastra laboratories).

Results

On physical examination, there were no abnormal abdominal findings. Other than slightly elevated γGTP (57 IU/l: normal 10–47) and blood glucose (107 mg/dl: normal 69–104) levels, all other laboratory tests, including the hematological profile, renal function, pancreatic enzymes, liver enzymes, electrolytes, and tumor markers (carbohydrate antigen (CA) 19-9 and carcinoembryonic antigen (CEA)) were within normal limits.

On gastroduodenoscopy, the stomach appeared normal, but there was a villous polypoid tumor, about 3 cm in diameter, with a stalk in the second portion of the duodenum (Fig. 1). The major duodenal papilla was identified about 2 cm distal to the tumor. Computed tomography (CT) demonstrated a solid 40×35-mm tumor with expansive growth that occupied the lumen of the descending portion of the duodenum and showed slight attenuation with contrast medium. Tumoral extension toward the underlying pancreas was not detected. The CT also showed a series of pancreatic stones within the duct of the head of the pancreas. The distal side of the pancreatic duct was dilated and the parenchyma of the distal pancreas showed mild atrophy. Neither lymph node involvement nor distant metastasis was detected. Magnetic resonance cholangiopancreatography (MRCP) (Fig. 2) and the coronal view of magnetic resonance image (MRI) showed the relationship between the stones and the pancreatic duct more clearly. The series of stones was about 3 cm long; one end of the stones appeared to be positioned near the stalk of the tumor, and the other end was within the main pancreatic duct. The stones were suspected to be located within the dorsal pancreatic duct. MRCP also revealed a short ventral pancreatic duct; a communication between the dorsal and ventral pancreatic duct was not clearly identified. These findings suggested the existence of pancreas divisum.

Pathological examination of the preoperative biopsy specimen revealed a well-differentiated adenocarcinoma. With a presumptive diagnosis of adenocarcinoma of the duodenum or minor duodenal papilla and chronic pancreatitis, the patient had surgery. At laparotomy, because the tumor had a thick and broad stalk on palpation, it was suspected that the tumor might have invaded the duodenum or the underlying pancreas. Consequently, a subtotal stomach-preserving pancreaticoduodenectomy (SSpPD) was performed.

Macroscopic examination showed a villous polypoid tumor, 50×30×25 mm in size; the stalk was 15 mm in diameter. The tumor was located about 2 cm proximal from the major papilla, which was normal in size and shape. The cut surface showed that the dorsal pancreatic duct was

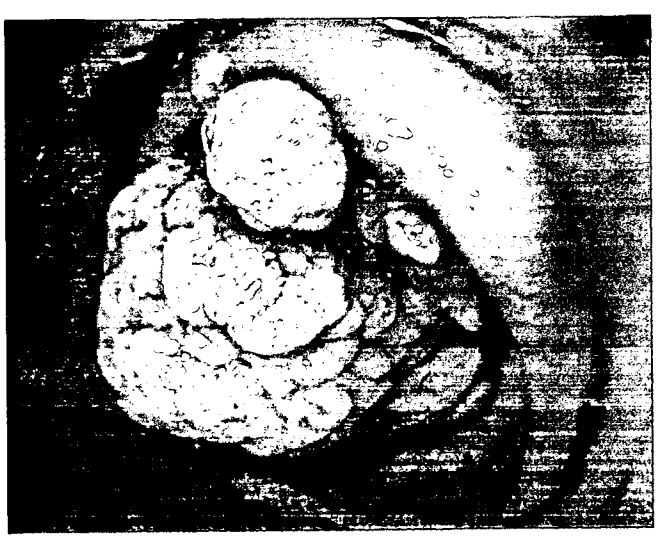


Fig. 1 Gastroduodenoscopy showing a villous polypoid tumor with a stalk in the second portion of the duodenum

Fig. 2 Magnetic resonance cholangiopancreatography (MRCP) showing a short ventral pancreatic duct (*white arrow*); the communication between the dorsal and ventral pancreatic ducts is not clearly identified. A series of stones (*white arrow head*) is seen in the dorsal pancreatic duct

obstructed by a series of stones, as had been demonstrated by the preoperative examinations (Fig. 3).

Microscopic examination showed that the main exophytic tumor was composed of eosinophilic tall columnar cells with oval and pseudostratified nuclei that were arranged in well-formed tubular pattern (Fig. 4a). The tumor was diagnosed as a well-differentiated tubular adenocarcinoma. The main tumor was limited to the mucosa and regarded as in-situ carcinoma without stromal invasion. At the tumor's stalk, muscular bundles similar to the sphincter of Oddi, which encircled the dorsal pancreatic duct, were present. These findings suggested that the tumor had arisen from the minor papilla and not from the duodenum. The adenocarcinoma cells spread through the sphincter bundles and the dorsal pancreatic duct; they replaced the normal pancreatic duct epithelium with intraductal carcinoma peripherally (Fig. 5a). Intraductal adenocarcinoma with micropapillary projection was observed in almost all areas of the dorsal pancreas, predominantly around the dorsal pancreatic duct. At the frontal edge of the intraductal spread, dysplastic epithelium and hyperplastic epithelium were observed (Figs. 5 and 6). At the cut end of the pancreas, the epithelium of the main pancreatic duct and the other branched ducts showed hyperplastic changes, which were considered to be reactive changes caused by tumor spread.

On immunohistochemical staining, both the main polypoid adenocarcinoma and the intraductal lesions showed the mixed positive pattern of CK7 and CK20. Muc-5AC was also multifocally positive both in the main tumor and the intraductal components. Muc-2 was negative except small number of cells in the base of the main tumor. The expression pattern for CK7, CK20, and Muc-5AC in the main tumor was maintained even in the ductal spreading area of the pancreas (Fig. 4).

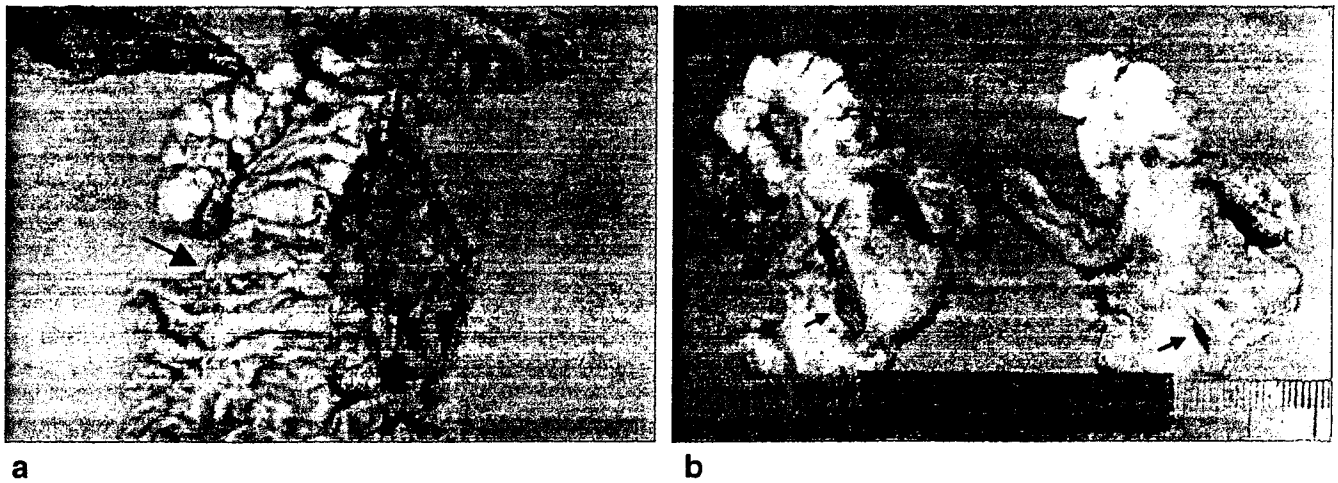
Chronic pancreatitis was found in the dorsal pancreatic parenchyma, with infiltration of inflammatory cells and fibrocollagenous tissue; the ventral pancreas was almost normal (Fig. 5a). The ventral pancreatic duct was short and narrow, and the epithelium of the ventral pancreatic duct did not include any carcinoma, dysplastic cells, or hyperplastic cells. These findings supported the presence of pancreas divisum. It is surprising to note that intraductal spread of carcinoma cells was observed in the small branches of the uncinate process, which anatomically belongs to the ventral pancreas, which was unaffected by pancreatitis (Figs. 5a and 6). About half of the uncinate process had carcinoma in situ. Neither lymphovascular invasion nor lymph node metastasis was observed.

The postoperative course was uneventful and the patient was discharged on the 13th postoperative day.

Discussion

The minor duodenal papilla is situated in the anterior duodenal wall, about 2 cm proximal to the major papilla [2, 16]. It primarily drains pancreatic fluid from the dorsal pancreas to the duodenum in the embryo [7].

Tumors of the minor duodenal papilla are uncommon, and few cases have been reported. Most reported cases have been submucosal benign tumors, such as carcinoid [12, 15, 19, 22, 23] and somatostatinoma [3, 13, 20]; only a single case of adenocarcinoma of the minor papilla has been previously reported [24]. Yamano et al. [24] reported a 77-year-old male with an ulcerating tumor, in which the dorsal pancreatic duct epithelium was partially replaced by carcinoma cells from the minor papilla; however, details of the pathological findings were not described. In contrast to our case, their case also had an intraductal papillary



a b

Fig. 3 **a** An exophytic polypoid tumor arising from the minor papilla. The major papilla (*arrow*) is normal in size and shape. **b** Cut section shows that the dorsal pancreatic duct was obstructed with a series of stones. (*arrows*; dorsal pancreatic duct with stones removed)

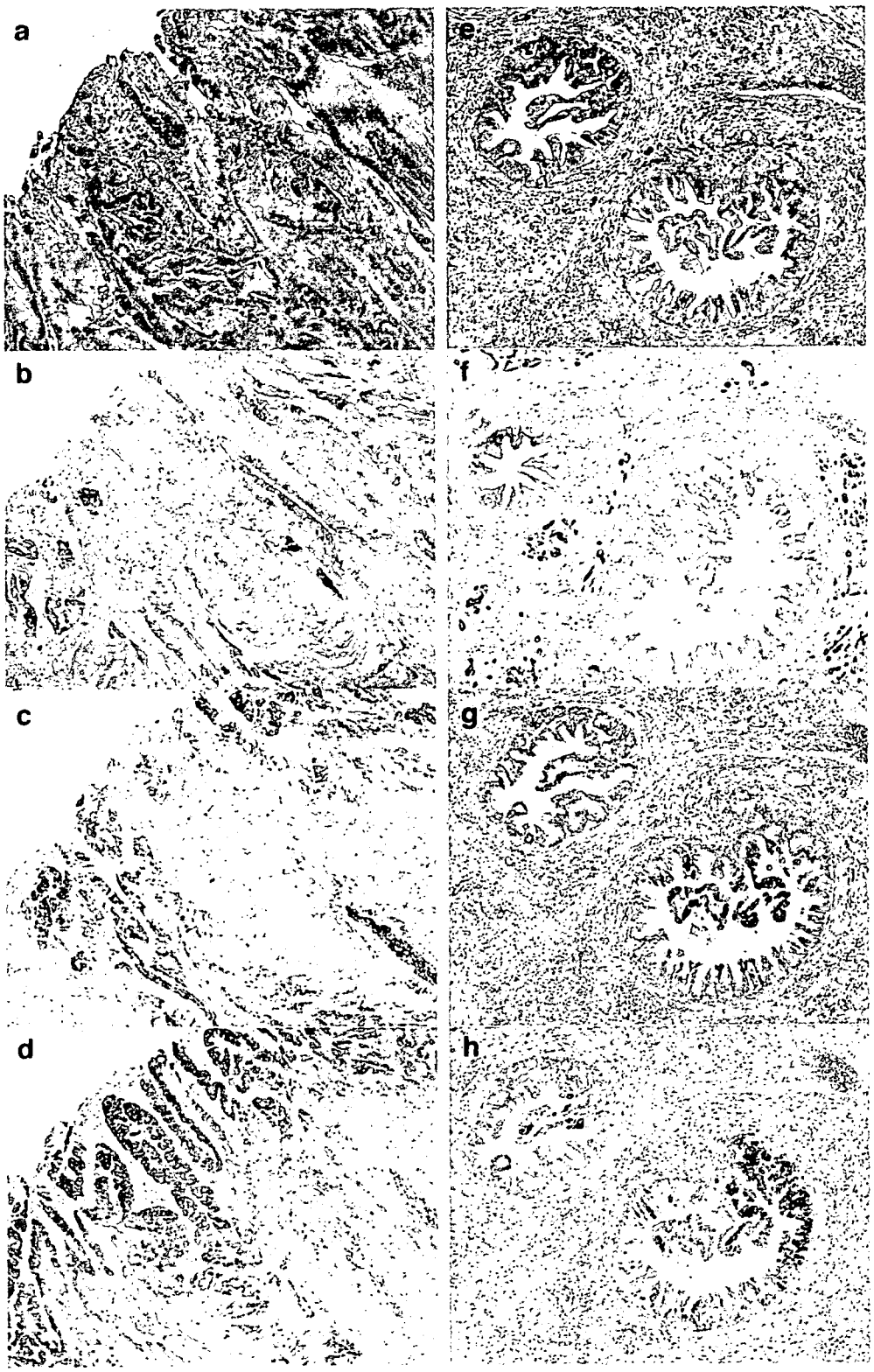


Fig. 4 **a–d** In-situ carcinoma of the main polypoid lesion (H&E, **a**) showing immunohistochemical positivity for CK7 (**b**), CK20 (**c**), and Muc-5AC (**d**) (×40). **e–h** Carcinoma in situ observed in the dorsal pancreatic duct (H&E, **e**) showing immunohistochemical positivity for CK7 (**f**), CK20 (**g**), and Muc-5AC (**h**) (×40)

adenoma with mucin hypersecretion in the cystically dilated branch duct in the head of the pancreas. Although the two tumors differed in their gross type, both cases had intraductal spread of the cancerous component. In the present case, however, carcinoma in situ had spread close to the cut end margin and also to the tiny peripheral branches without cystic dilatation.

In addition, our case had calcified stones located in the dorsal pancreatic duct. The majority of cases of pancreatic stones are secondary to chronic pancreatitis; however, the

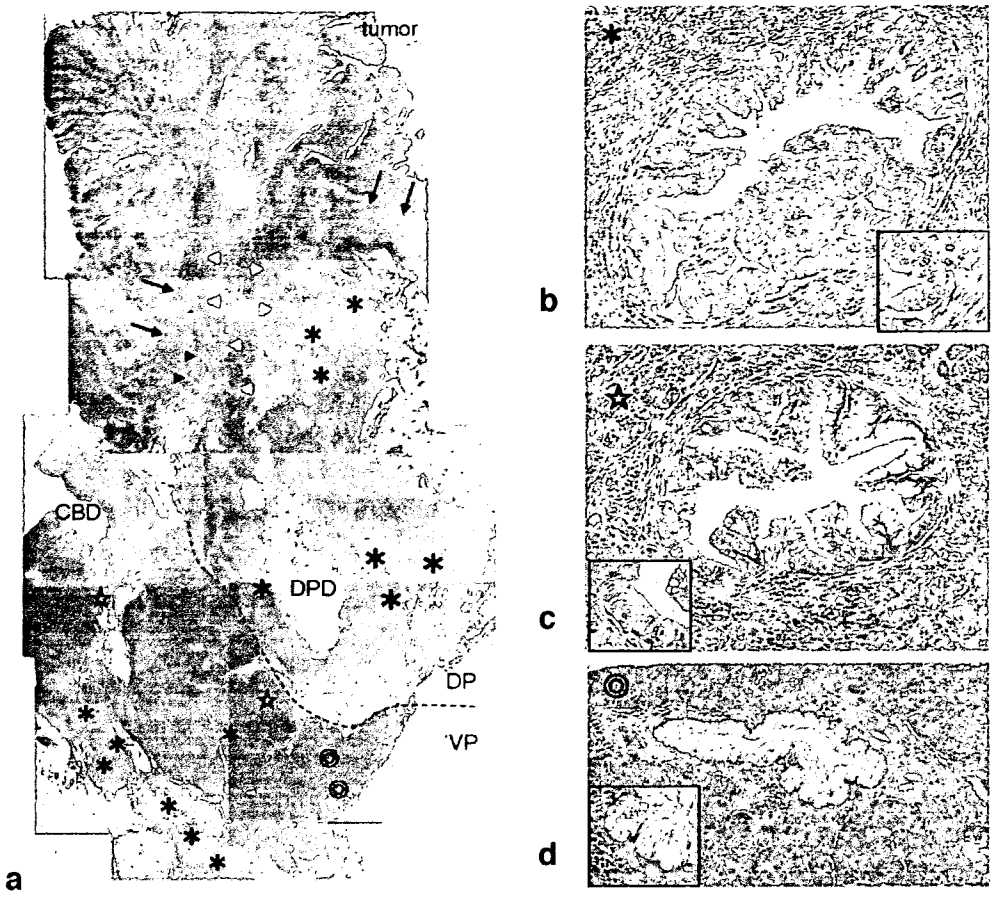


Fig. 5 **a** At the stalk of the tumor, muscular bundles (*black arrow* heads), which encircle the dorsal pancreatic duct (*white arrow heads*), are present. The carcinoma in situ has spread through the sphincter bundles to the branched duct around the dorsal pancreatic duct (DPD); dysplastic and hyperplastic epithelium are present peripherally (*black arrows*, muscular layer of the duodenum; *, carcinoma in situ; ☆, dysplastic epithelium; ◎, hyperplastic epithelium; *CBD*, common bile duct). Chronic pancreatitis is evident in the dorsal pancreas (DP), while the ventral pancreas (VP) appears almost normal. **b** Carcinoma in situ (*) (H–E×100). **c** Dysplastic epithelium (☆) (H–E×100). **d** Hyperplastic epithelium (◎) (H–E×100)

size, number, and distribution of stones vary by the type of pancreatitis [5, 10, 18]. In alcoholic chronic pancreatitis, there are numerous irregular small stones throughout the pancreas. On the other hand, in obstructive pancreatitis, the stone is usually large, solitary, and inside the lumen of the pancreatic duct. The pathogenesis of the stone is considered to be because of the stasis of pancreatic flow [17, 25]. With respect to stones in patients without a history of alcohol abuse, stagnation of pancreatic fluid as a consequence of the duct obstruction by a tumor might lead to development of stones, as in the present case.

The MRCP and MRI findings and the distribution of pancreatitis, which mainly affected the dorsal pancreas, suggested the existence of pancreas divisum. Pancreas divisum is a common congenital anomaly of the pancreas, which results from an abnormal fusion between the ventral and dorsal

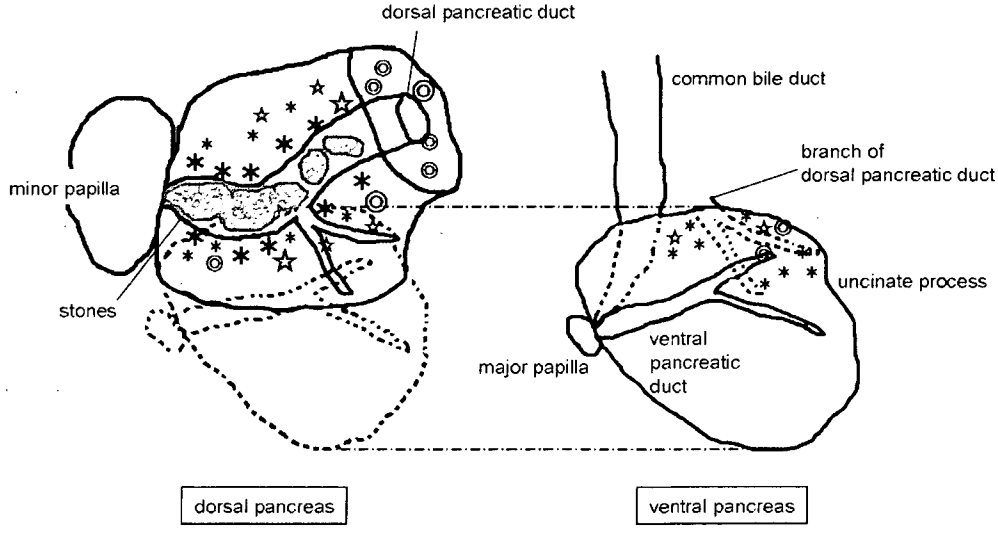


Fig. 6 Schematic distribution of carcinoma in situ (*), dysplastic epithelium (☆), and hyperplastic epithelium (◎). The dorsal and ventral pancreases are depicted separately for convenience. Carcinoma in situ is located predominantly near the dorsal pancreatic duct, and the dysplastic and hyperplastic epithelium is observed surrounding the carcinoma in situ. The branches of the uncinate process, which belongs to the ventral pancreas, are partially involved with cancerous spread

pancreatic ducts during fetal development [8]. It is divided into complete and incomplete types. In the complete type, there is no communication between the two ducts, whereas in the incomplete type, an inadequate communication exists between the two ducts. Pancreas divisum is strongly associated with pancreatitis, especially in alcoholic patients. Irrespective of the type of pancreas divisum, pancreatitis often occurs only in the dorsal pancreas, as was observed in this case. An interaction between a poorly functioning minor duodenal papilla and the increased flow of pancreatic juice caused by alcohol or food intake is thought to cause pancreatitis [8]. The presence of this anomaly in our patient may have promoted stasis of the pancreatic juice and the formation of the pancreatic stones.

In the present case, most of the intraductal carcinoma was limited to the dorsal pancreas, though some carcinoma was observed in the branches of the uncinate process, which is anatomically classified as being part of the ventral pancreas. In complete pancreas divisum, intraductal carcinoma in the dorsal pancreas never spreads to the ventral pancreas. Because intraductal components were observed in the uncinate branches, minor peripheral communications must have existed between the two ducts in our patient.

Pathologically, adenocarcinoma of the major papilla is classified into two types: intestinal and pancreatobiliary type, based on the epithelium of its origin [1, 9]. The former is derived from intestinal (duodenal) mucosa covering the papilla, whereas the latter is associated with pancreatobiliary epithelium lining the common channel and duct systems within the papilla. This classification is also supported by immunohistochemical staining such as cytokeratin and apomucin [26]. CK20 and Muc-2 are associated with the intestinal type, whereas CK7 and Muc-5AC expression is relatively specific for the pancreatobiliary type. Immunohistochemical staining in the present case showed the mixed positive expression for CK7 and CK20. This finding indicated that the tumor might arise from the transitional area between the intestinal mucosa covering the minor papilla and the dorsal pancreatic ductal epithelium. Moreover, the mixed positive pattern for CK7 and CK20 was observed uniformly from the main polypoid adenocarcinoma to the minute intraductal lesions, suggesting that the character of both components was equivalent. This fact supported that the intraductal components were extended from the main lesion.

On microscopic examination, the morphological feature of the intraductal carcinoma component was similar to that seen in intraductal papillary mucinous neoplasm (IPMN) of the pancreas or pancreatic intraepithelial neoplasia (PanIN). IPMN generally demonstrates grossly visible cystic lesions or exophytic masses (usually >1 cm in diameter) along with grossly visible papillae and luminal mucin production [6]. In the present case, the lesion suggesting IPMN was not detected in either radiological or macroscopical examination, and moreover, mucin was not observed on the cut surface of the specimen. In addition, the main polypoid tumor conclusively involved the minor papilla. The intraductal component, which was observed even in the tiny peripheral branches (<5 mm) with lowering the grade of the lesion distally, was in direct continuity with the main tumor. Taken together, it is consistent that the association of IPMN or PanIN was less likely, and the tumor originated from the minor papilla, then extended into the distal pancreatic duct.

The pathogenesis of our case likely involved the following factors: (1) the patient had a congenital, incomplete type of pancreas divisum that had been asymptomatic; (2) the tumor arose in the minor papilla and obstructed the dorsal pancreatic duct; (3) stagnation of the pancreatic fluid from the dorsal pancreas caused pancreatitis and the formation of calcified stones; (4) on the other hand, the intraductal tumor extended widely into the peripheral ducts, and some tumor components traveled through small communications between the dorsal and ventral pancreatic ducts, and eventually reached to the branches of the uncus, which was unaffected by pancreatitis.

It is interesting to note that, in this case, the intraductal component of the adenocarcinoma of the minor papilla extended along the pancreatic duct more than expected; it extended even beyond the minor communication between the dorsal and ventral pancreatic ducts of pancreas divisum, which was detected incidentally.

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