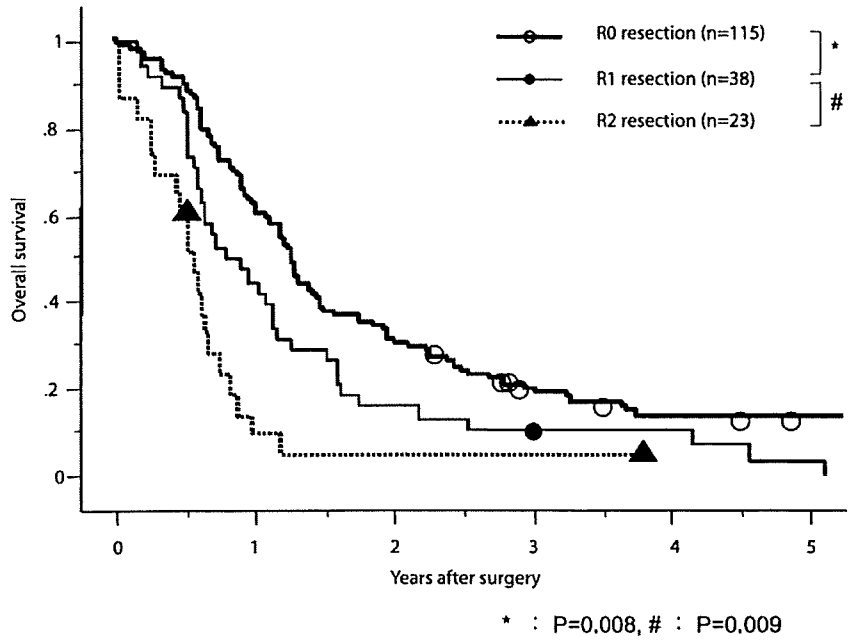

FIGURE 1. Comparison of OS curves for patients with pancreatic head cancer who underwent PD or PPPD according to the resection margin status. The median survival for 115 patients with R0 resection was 15.2 months, which was significantly longer ($P = 0.008$) than that for 38 patients with R1 resection (9.4 months). The median survival for 23 patients with R2 resection (6.2 months) was significantly shorter ($P = 0.009$) than that of R1 resection patients.

anterior pancreatic tissue invasion (OR, 2.50; 95% CI, 1.18–5.56) were independently associated with positive resection margins (Table 2). When patients with R2 resection were excluded from the analysis, only extrapancreatic nerve plexus invasion was independently associated with R1 resection (OR, 7.17; 95% CI, 3.01–17.21; Table 3).

Survival Analysis of Clinicopathologic Factors in Patients Who Underwent PD/PPPD for Pancreatic Head Cancer

The 1-year, 3-year, and 5-year OS rates of 176 patients were 51.5 %, 15.7 %, and 9.9 %, respectively, with a median OS time of 12.3 months. The median OS in patients with R0, R1, and R2 resections were 15.2 months, 9.4 months, and 6.2 months, respectively. The patients with R1 resection showed better OS compared with patients with R2 resection ($P = 0.009$) and worse OS than R0 resection patients ($P = 0.008$; Fig. 1). Univariate analysis of factors affecting OS showed that invasion of cancer cells into the anterior pancreatic tissue, retropancreatic tissue, bile duct, duodenum, portal venous system, arterial system, extrapancreatic nerve plexus, lymph vessels, blood vessels, and intrapancreatic nerves as well as lymph node metastasis, histological type (other than well-differentiated tubular adenocarcinoma and papillary adenocarcinoma), tumor size larger than 2 cm in diameter, and positive resection margins (either R1 or R2) were all associated with poor prognosis (Table 4). By multivariate analysis, lymph node metastasis (hazard ratio [HR], 1.77; 95% CI, 1.15–2.73), portal venous system invasion (HR, 1.53; 95% CI, 1.03–2.28), extrapancreatic nerve plexus invasion (HR, 1.54; 95% CI, 1.03–2.29), and R2 resection (HR, 3.52; 95% CI, 1.97–6.26) were identified as factors that independently affected OS (Table 5). When R1 resection was included as a covariate for analysis instead of R2 resection, R1 resection was not independently influential (HR, 0.97; 95% CI, 0.60–1.54). Lymph node metastasis (HR, 1.92; 95% CI, 1.21–3.05), portal venous system invasion (HR, 1.74; 95% CI, 1.13–2.70), and extrapancreatic nerve plexus invasion (HR, 1.61; 95% CI, 1.01–2.55) remained significant prognostic factors (Table 6).

DISCUSSION

Many reports have referred to clinicopathologic factors influencing survival after resection for pancreatic cancer.[6–14,18] Whether a positive resection margin independently affects survival has always been a matter of controversy.[12–14] The conflict may be due to the differences in the study design (ie, whether the cases with R2 resection were included or not) or in the system used for the pathologic examination of the resected specimens.[19] In the recent literature, Raut et al[14] reviewed published studies and reported that R1 rates and R1 + R2 rates were 17% to 85% and 17% to 45%, respectively.

In the present study, we evaluated the resection margin status both macroscopically and microscopically and then assigned the patients into R0, R1, and R2 resection categories. As a result, 115 (65.3 %) of the 176 patients were evaluated as R0 resection, whereas 38 patients (21.6%) were evaluated as R1, and 23 patients (13.1%) as R2 resection. These rates did not deviate from the previously reported R1/R1 + R2 rates. This means that complete resection was not achieved in one third of patients who underwent PD/PPPD, despite extended pancreatectomies accompanied by portal vein resection and extrapancreatic nerve plexus dissection being performed. As a high-volume center, several of our cases were referred from other hospitals after being deemed unresectable because of locally advanced disease. A high percentage of noncurative resection would therefore seem inevitable. Indeed, the condition of the 107 (60.8%) of the 176 patients was diagnosed as pT4 under the classification of the JPS[17] on the ground that cancer cells invaded into the portal venous system, arterial system, extrapancreatic nerve plexus, or adjacent organs, and the proportion of patients receiving portal venous resection was unusually high. Among other studies

TABLE 4. Univariate Analysis of Factors Affecting OS After Surgery in Patients With Pancreatic Head Cancer

	No. Patients	OS Rates (%) 1 YR	3 YR	5 YR	P
Total patients	176	51.5	15.7	9.9	
Age					
≥60 yr	122	50.5	14.1	8.5	0.318
<60 yr	54	53.7	19.9	13.7	
Sex					
M	115	53.0	16.9	10.1	0.438
F	61	48.5	13.4	9.7	
Tumor size					
<2 cm	26	80.8	38.1	28.6	<0.001*
≥2 cm	148	46.3	12.0	7.1	
Anterior pancreatic tissue invasion					
Negative	103	62.3	20.0	12.9	<0.001*
Positive	73	36.3	9.8	5.9	
Retropancreatic tissue invasion					
Negative	60	68.3	22.2	17.8	<0.001*
Positive	116	42.7	12.2	5.6	
Bile duct invasion					
Negative	48	58.3	26.8	21.1	0.041*
Positive	128	48.9	11.6	6.0	
Duodenal invasion					
Negative	76	57.4	20.9	13.9	0.019*
Positive	100	47.0	12.0	7.0	
Portal venous system invasion					
Negative	91	64.8	23.7	14.9	<0.001*
Positive	85	37.0	7.2	4.8	
Arterial system invasion					
Negative	159	54.5	16.8	11.2	0.049*
Positive	17	23.5	5.9	0	
Extrapancreatic nerve plexus invasion					
Negative	121	58.9	20.6	13.5	<0.001*
Positive	56	35.7	5.4	0	
Lymph vessel invasion					
Negative	19	88.9	36.7	30.6	<0.001*
Positive	151	46.4	12.6	7.1	
Venous invasion					
Negative	82	64.3	20.4	15.4	<0.001*
Positive	88	38.6	10.2	5.1	
Perineural invasion					
Negative	25	75.3	44.9	39.2	<0.001*
Positive	145	47.6	10.2	5.2	
Lymph node metastasis					
Negative	53	71.2	30.5	22.6	<0.001*
Positive	123	43.1	9.6	5.1	
Histological differentiation					
Well/papillary	30	82.8	40.5	30.9	<0.001*
Others	143	46.2	11.1	6.1	
Radiation therapy					
IORT (+)	112	50.0	10.5	5.6	0.098
IORT (−)	64	54.1	25.3	17.5	
Chemotherapy					
Yes	77	58.4	16.7	10.8	0.223
No	91	45.7	14.5	8.3	
Chemotherapy with gemcitabine					
Yes	34	47.1	17.6	17.6	0.724
No	142	52.6	15.4	9.2	
Resection margin status					
R0	115	61.7	19.5	13.2	0.008*
R1	38	44.7	10.5	3.5	
R2	23	9.4	4.7	—	0.009*

*Statistically significant.

looking at the issue of resection margin, the cohort analyzed in the current study can be characterized as including a high proportion of locally advanced and challenging cancer treated with an aggressive policy toward complete resection.

We next analyzed the association of clinicopathologic characteristics with resection margin status and found that extrapancreatic nerve plexus, anterior pancreatic tissue, and retropancreatic tissue invasions were independently associated with positive resection margins (R1 and R2), whereas only extrapancreatic nerve plexus invasion was found to independently associate with R1 resection. Pancreatic head cancer often invades the extrapancreatic nerve plexus.[20,21] Indeed, 56 (31.8%) of the 176 patients in our series had microscopic invasion of cancer cells into the nerve plexus. Preoperative diagnosis of extrapancreatic nerve plexus invasion remains challenging, although the detection of soft tissue density behind the SMV or adjacent to the superior mesenteric artery (SMA) with multi–detector row CT may be a clue to its diagnosis.[22] Intraportal endovascular ultrasonography has been used to

TABLE 5. Multivariate Analysis of Factors Affecting OS After Surgery in Patients With Pancreatic Head Cancer (R2 Resection Was Included as a Covariate)

Covariate	HR	95% CI	P
Tumor size (≥2 cm)	1.21	0.66–2.21	0.531
Anterior pancreatic tissue invasion	0.98	0.66–1.46	0.912
Retropancreatic tissue invasion	1.06	0.71–1.58	0.785
Bile duct invasion	1.06	0.70–1.61	0.785
Duodenal invasion	1.29	0.84–1.98	0.247
Portal venous system invasion	1.53	1.03–2.28	0.035*
Arterial system invasion	0.76	0.40–1.47	0.420
Extrapancreatic nerve plexus invasion	1.54	1.03–2.29	0.034*
Lymph vessel invasion	0.97	0.48–1.96	0.930
Venous invasion	1.05	0.71–1.56	0.810
Perineural invasion	1.69	0.86–3.34	0.131
Lymph node metastasis	1.77	1.15–2.73	0.010*
Histological grade of differentiation†	1.60	0.95–2.69	0.076
Macroscopically positive margin; R2	3.52	1.97–6.26	<0.001*

*Statistically significant.

†Other than well-differentiated tubular adenocarcinoma and papillary adenocarcinoma.

TABLE 6. Multivariate Analysis of Factors Affecting OS After Surgery in Patients With Pancreatic Head Cancer (R1 Resection Was Included as a Covariate)

Covariate	Relative Hazard	95% CI	P
Tumor size (≥2 cm)	1.37	0.74–2.54	0.311
Anterior pancreatic tissue invasion	0.94	0.61–1.46	0.783
Retropancreatic tissue invasion	0.95	0.62–1.46	0.824
Bile duct invasion	0.98	0.63–1.53	0.928
Duodenal invasion	1.10	0.70–1.74	0.685
Portal venous system invasion	1.74	1.13–2.70	0.013*
Arterial system invasion	0.90	0.39–2.05	0.797
Extrapancreatic nerve plexus invasion	1.61	1.01–2.55	0.046*
Lymph vessel invasion	1.00	0.49–2.04	0.998
Venous invasion	0.98	0.64–1.50	0.923
Perineural invasion	1.82	0.91–3.64	0.089
Lymph node metastasis	1.92	1.21–3.05	0.006*
Histological grade of differentiation†	1.58	0.90–2.77	0.113
Microscopically positive margin, R1	0.97	0.60–1.54	0.882

*Statistically significant.

†Other than well-differentiated tubular adenocarcinoma and papillary adenocarcinoma.

diagnose not only portal vein invasion but also nerve plexus invasion during surgical exploration in our department.[23] On the basis of the findings with multi–detector row CT and/or intraportal endovascular ultrasonography, we have been performing complete or right semicircular dissection of the nerve plexus around the SMA along with the extrapancreatic nerve plexus to obtain a negative resection margin.[3] This policy can be justified from the viewpoint of the curability of the pancreatectomy, considering a strong association of nerve plexus invasion with positive resection margin presented in the present study. But it is still unclear whether this strategy can provide survival benefit to the patients undergoing PD/PPPD for pancreatic cancer.

To elucidate the significance of resection margin status as a prognostic factor, we conducted univariate and multivariate analyses of factors affecting OS. The OS in patients with R1 resection (median survival [MS], 9.4 months) was shorter than in patients with R0 resection (MS, 15.2 months) and was longer than in patients with R2 resection (MS, 6.2 months). The differences in survival between R0 and R1 and between R1 and R2 were statistically significant by the log-rank test ($P = 0.008$ and $P = 0.009$, respectively). This result indicates that although patients with a positive resection margin (R1 + R2, n = 61) rarely achieved longtime survival (5-year survival rate was 2.8%, MS, 7.6 months), a surgery with microscopically positive margin (R1 resection) might have some positive impact on survival when compared with a surgery with grossly affected margin (R2 resection). By multivariate analysis of variables that were confirmed to possibly affect the survival in the univariate analysis, R2 resection was identified as an independent factor affecting OS together with lymph node metastasis and portal venous system and extrapancreatic nerve plexus invasions. When R2 resection was replaced by R1 resection in the same analysis, however, R1 resection was found not to be an independent predictor of poor outcome, whereas lymph node metastasis and portal venous system and extrapancreatic nerve plexus invasions remained significant as indicators of poor prognosis. These results indicate that although grossly positive resection margin adversely affected the survival, microscopic residual disease in the resection margin did not influence the survival significantly after PD/PPPD for pancreatic cancer. The cases with R1 resection were more liable to have lymphatic involvement than R0 resection cases, and this might have been more important as a cause for poor prognosis. This remains a mere speculation because precise data on the pattern of recurrence were not available.

In summary, the present study shows that R0 resection achieved through an aggressive policy toward complete resection of pancreatic cancer had a significant impact on survival when compared with R2 resection. R1 resection was not a significant indicator of poor prognosis and was in sharp contrast with R2 resection that, together with lymph node metastasis, portal venous system invasion, and nerve plexus invasion, was found by multivariate analysis to adversely influence the survival. The patients treated with R1 resection actually lived longer than those who underwent R2 resection, although the survival time was rarely longer than 5 years. Thus, extended pancreatectomy for locally advanced pancreatic head cancer should be applied only in cases where R2 resection can be avoided.

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Pancreatic head resection with segmental duodenectomy for pancreatic neoplasms

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Abstract
Background/purpose We have experienced 67 cases of pancreatic head resection with segmental duodenectomy (PHRSD) for benign or low-grade malignant tumor of the pancreatic head region. Here we introduce our operative technique for these 67 cases.
Methods Pancreatic head resection is performed with segmental duodenectomy including minor and major papilla. By conserving the right gastric artery and the gastroduodenal artery, 5–7 cm of the first portion of the duodenum is preserved with good arterial circulation. In addition, by conserving the anterior inferior pancreatoduodenal artery, the third portion and anal side or the second portion of the duodenum are preserved with good arterial circulation. Cholecystectomy is performed. The procedure is completed by resection of the pancreatic head with 3–4 cm of segmental duodenectomy including minor and major papilla. Reconstruction of the alimentary tract is performed with pancreatogastrostomy, end-to-end duodenoduodenostomy and end-to-side choledochoduodenostomy.
Results In 67 cases with diseases of the pancreatic head region, chiefly intraductal papillary mucinous neoplasms, this procedure was successfully performed without operative or hospital death. Postoperative quality of life was quite satisfactory.
Conclusion Total resection of the pancreatic head can be performed safely and effectively by this procedure.

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Keywords Pancreatic head resection with segmental duodenectomy · Organ-preserving pancreatectomy · Pancreatogastrostomy · Intraductal papillary mucinous neoplasms of pancreatic head

Introduction

Organ-preserving pancreatic resections are reasonable surgical options for benign or low-grade malignant tumors of the pancreas. Pylorus-preserving pancreatoduodenectomy (PpPD) [1] has now been recognized as the ideal surgical method for treating benign, low-grade malignancy and malignant tumors of the pancreatic head region. Duodenum-preserving pancreatic head resection (DpPHR) [2] is also one of the options for organ-preserving pancreatic head resection. In the DpPHR, there are two types of operation: combined resection of the common bile duct and common bile duct preservation [2–4]. In DpPHR the arterial blood circulation of duodenum or common bile duct is a great problem. Ischemia of the duodenum, or common bile duct, causes necrosis of the duodenum or common bile duct and leads to perforation [3, 4]. The other major problem with DpPHR and partial resection of the pancreatic head is failure to complete extirpation of intraductal papillary mucinous neoplasms (IPMN), because IPMN tends to spread into the main or branch pancreatic ducts. To prevent these complications, we have been performing complete pancreatic head resection with segmental duodenectomy (PHRSD) [5–7], including the minor and major papilla, for mainly benign or low-grade malignant tumors of the pancreatic head region in 67 cases. Reconstruction of the alimentary tract after PHRSD has been performed with pancreatogastrostomy, end-to-end duodenoduodenostomy

and end-to-side choledochoduodenostomy. We report here the operative procedure of PHRSD and postoperative results.

Patients and methods

From 1988 to 2008, 67 patients who underwent PHRSD had 47 IPMNs, 7 non-functional endocrine tumors of the pancreatic head region, 6 papilla of Vater cancers, 2 serous cytadenomas, 1 pancreas head cancer, 1 common bile duct cancer, 1 insulinoma, 1 annular pancreas and 1 anomalous engagement of the pancreatobiliary ductal system. Laparotomy is done by upper midline skin incision. The gastrocolic and duodenocolic ligaments are divided with preservation of the right gastroepiploic artery (RGEA) and vein to explore the front of the pancreas. The right gastroepiploic vein is ligated and divided at the root. The anterior–superior pancreatoduodenal artery (ASPDA), the posterior–superior pancreatoduodenal artery (PSPDA) and a few other branches running from the gastroduodenal artery (GDA) towards the pancreas are ligated and divided. By conserving the RGEA and GDA, 5–7 cm of the first portion of the duodenum is preserved with good arterial circulation. The pancreas is divided on the line of the portal vein. The extrapancreatic nerve plexus between the uncinate process and the superior mesenteric artery is preserved, so the inferior pancreatoduodenal artery (IPDA) is preserved. The anterior–inferior pancreatoduodenal artery (AIPDA) is preserved, and the posterior–inferior pancreatoduodenal artery (PIPDA) is ligated and divided. The AIPDA is ligated and divided near the major papilla (Figs. 1, 2). Cholecystectomy is performed. The common bile duct is divided at the upper border of the pancreas. A 2–3 cm ischemic area of the duodenum, including the major and minor papilla, is observed (Fig. 3). The oral side of the duodenum is divided 5–7 cm from the pyloric ring. The anal side of the duodenum is divided at the point of AIPDA ligation. Thus, PHRSD with preservation of GDA is completed. The length of the resected duodenum ranges from 3 to 5 cm (Fig. 2). Reconstruction of the alimentary tract is performed with pancreatogastrostomy (temporary pancreatic stent in the main pancreatic duct of the remnant pancreas and drained externally), end-to-end duodenoduodenostomy, and end-to-side choledochoduodenostomy (temporary transhepatic biliary stenting) (Fig. 4).

Results

No operative or hospital death was observed in the 67 cases. Minor leakage from the anastomosis portion of alimentary tract such as pancreatogastrostomy in 19.4%,

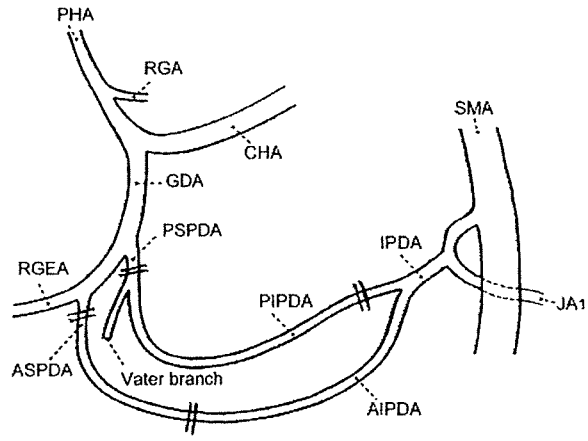

Fig. 1 Divided lines of the pancreatoduodenal arteries in pancreatic head resection with segmental duodenectomy. *PHA* proper hepatic artery, *RGA* right gastric artery, *CHA* common hepatic artery, *GDA* gastroduodenal artery, *RGEA* right gastroepiploic artery, *PSPDA* posterior–superior pancreatoduodenal artery, *ASPDA* anterior–superior pancreatoduodenal artery, *IPDA* inferior pancreatoduodenal artery, *PIPDA* posterior–inferior pancreatoduodenal artery, *AIPDA* anterior–inferior pancreatoduodenal artery, *JA1* first jejunal artery, *SMA* superior mesenteric artery

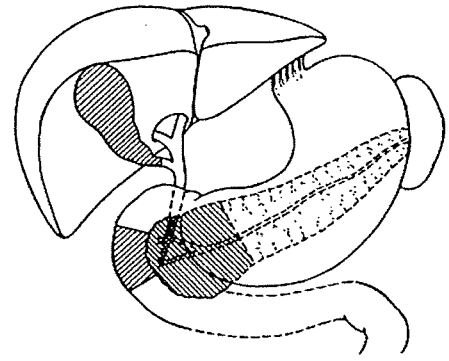

Fig. 2 Resected portion in pancreatic head resection with segmental duodenectomy

choledochoduonenostomy in 4.5% and duodenoduodenostomy in 1.5% were observed, but healed with conservative treatment. Intraabdominal bleeding was observed in two cases, but successfully treated by transarterial embolization. All patients discharged from the hospital showed extremely good postoperative quality of life (QOL).

Discussion

Organ-preserving pancreatic resection for benign tumor of the pancreatic head or chronic pancreatitis such as PpPD [1] or DpPHR [2] has been recognized as the ideal surgical method. There are two types of DpPHR operation: combined resection of the common bile duct [3] and

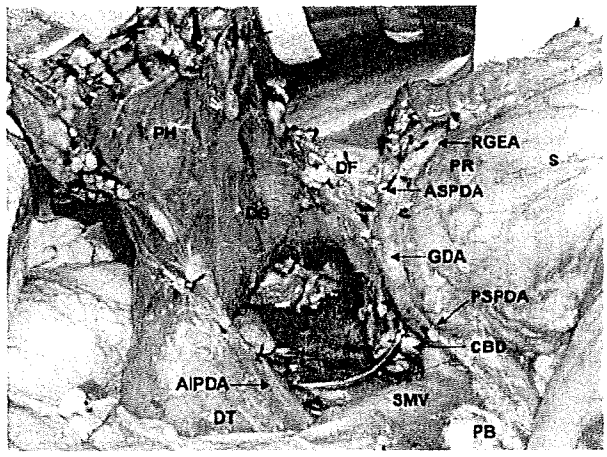

Fig. 3 Segmental duodenectomy completes the total pancreatic head resection. *PH* pancreatic head, *PB* pancreatic body, *DF* duodenal first portion, *DS* duodenal second portion, *DT* duodenal third portions, *S* stomach, *PR* pyloric ring, *CBD* common bile duct, *GDA* gastroduodenal artery, *PSPDA* posterior–superior pancreatoduodenal artery, *ASPDA* anterior–superior pancreatoduodenal artery, *RGEA* right gastroepiploic artery, *SMV* superior mesenteric vein

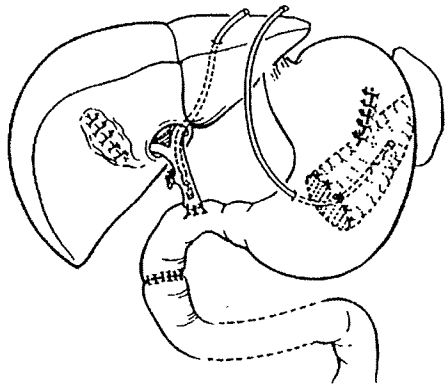

Fig. 4 Schematic of alimentary tract reconstruction after pancreatic head resection with segmental duodenectomy

preservation of the common bile duct [2, 4]. To preserve the duodenum and common bile duct, the preservation of the pancreatic head arcade of the arteries is very important. The anatomy of the arcade of the arteries of the pancreatic head has been studied [8, 9]. The branch of the PSPDA that runs along the right side of the common bile duct and toward the major papilla (Vater branch) is important to preserve the common bile duct and major papilla [8, 9], but this branch is difficult to visualize during operation. The preservation of the pancreatic parenchyma between the common bile duct and duodenum (groove area) is necessary to preserve this branch in DpPHR with the preservation of the common bile duct and sphincter function of major papilla [9]. The preservation of the anterior arcade of the arteries in the pancreatic head is technically difficult near the minor and major papilla. If these arteries cannot be preserved, postoperative ischemic necrosis or perforation of the common bile duct or duodenum may result [10, 11]. Successful complete resection of the pancreatic head with preservation of the common bile duct and duodenum has been reported [10, 11]. However, complete resection of the pancreatic head including the pancreatic parenchyma between the common bile duct and duodenum will cause ischemia of the common bile duct and major papilla. However, complete preservation of the arcade of the arteries of the pancreatic head with common bile duct preservation is technically difficult and impossible. DpPHR with incomplete resection of the pancreatic head cannot ensure complete extirpation of IPMN, because IPMN tends to spread into the main or branch ducts. High morbidity and mortality rates were observed in DpPHR [12]. We have already reported the advantage of PHRSD compared with PpPD in delayed gastric emptying, endocrine function, body weight decrease and postoperative enzyme substitution [7]. We recommend PHRSD for the above reasons.

Conclusions

PHRSD is a safe and reasonable technique appropriate for selected patients with benign or low-grade malignant tumor of the pancreatic head region, especially with benign or noninvasive IPMN.

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A history of smoking is inversely correlated with the incidence of gemcitabine-induced neutropenia

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Background: Smoking may affect the efficacy of chemotherapy and the incidence of adverse events. We investigated the correlation between smoking history and gemcitabine-induced neutropenia.

Patients and methods: Data on smoking history and incidence of grade 3–4 neutropenia were retrospectively gathered for 103 chemo-naive patients treated with gemcitabine monotherapy (59 patients with pancreatic, 41 with hepatobiliary and three with other cancers).

Results: There was a significantly higher incidence of grade 3–4 neutropenia among patients without a history of smoking (55.7%) than among those with a history of smoking (including current and ex-smokers; 23.6%) [odds ratio (OR) 0.244, 95% confidence interval (CI) 0.105–0.569; $P < 0.001$]. After adjustment for age, gender, platelet and baseline neutrophil counts, history of surgery for primary cancer, creatinine concentration, hemoglobin concentration, aspartate aminotransferase concentration, alanine aminotransferase concentration and total bilirubin concentration, logistic regression analysis identified a history of smoking as an independent inverse predictor of gemcitabine-induced neutropenia (OR 0.188, 95% CI 0.057–0.618; $P = 0.006$).

Conclusion: Patients without a history of smoking may be at higher risk of developing gemcitabine-induced neutropenia. The mechanism underlying this phenomenon is unclear at this point.

Key words: adverse effects, chemotherapy, gemcitabine, neutropenia, smoking

introduction

Gemcitabine is a deoxycytidine analogue that is widely used for many solid tumors as a single agent or in combination with other anticancer drugs [1–4]. The recommended dosage regimen for gemcitabine monotherapy consists of a 4-week cycle with 1000 mg/m^2 doses administered over 30 min on days 1, 8 and 15 [1, 5]. This dosage regimen is supported by the results of a Japanese phase I study involving pancreatic cancer patients [6]. However, in daily practice, we often encounter patients who cannot tolerate the recommended 1000 mg/m^2 dose of gemcitabine because of hematological adverse events, especially neutropenia. For some patients, it has been necessary to reduce the dose of gemcitabine to less than half the recommended dose because of gemcitabine-induced neutropenia.

Several enzymes are known to be involved in gemcitabine metabolism [7]. A recent study demonstrated that single-nucleotide polymorphisms (SNPs) in the cytidine deaminase (CDA) gene, which encodes a key enzyme in gemcitabine inactivation, influence the pharmacokinetics and toxicity of gemcitabine [8]. Considering the low allele frequencies of these SNPs, however, it seems that one or more unknown factors affect the pharmacokinetics and toxicity of gemcitabine. Smoking has been identified as a factor that potentially affects the pharmacokinetics of several anticancer drugs. Recent studies have demonstrated that smoking significantly lowers exposure to irinotecan and reduces the risk of irinotecan-induced neutropenia, at least in part by modulating irinotecan metabolism [9], and decreases the blood level of erlotinib by affecting erlotinib clearance rates [10, 11]. These findings prompted us to perform the current study to investigate the potential correlation between a history of smoking and gemcitabine-induced neutropenia.

patients and methods

patients

From November 2003 to November 2007, 103 chemo-naive patients underwent gemcitabine monotherapy at Kyoto University Hospital. For these patients, we retrospectively obtained data on smoking history and

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grade 3–4 gemcitabine-induced neutropenia from the hospital's electronic medical records system (CyberOncology®, CyberLaboratory Inc., Tokyo, Japan) [12]. Hematological adverse events were graded according to the Common Terminology Criteria for Adverse Events version 3.0 [13]. All patients provided written informed consent for the use of clinical data in the medical records system for the purposes of research. This study was approved by the Ethics Committee of Kyoto University Graduate School of Medicine (E-377).

treatment

Gemcitabine monotherapy was initiated at doses of 460–1000 mg/m^2 administered over 30 min on days 1, 8 and 15 over a 4-week cycle. The initial gemcitabine dose was adjusted at the discretion of individual physicians according to baseline bone marrow function, liver function, age and the risk of infection. For each cycle, if necessary, the dose and schedule of gemcitabine administration were adjusted in response to adverse events (hematological and otherwise) observed during the previous cycle. The most common cause of dose adjustment was hematological toxicity, especially neutropenia. Granulocyte colony-stimulating factor (G-CSF) was used for 11 patients.

smoking history

Information about the patients' smoking history was retrieved from electronic medical records based on information recorded during patient interviews. Patients were classified into smoking and non-smoking groups based on this information. The non-smoking group comprised those who stated that they had never been smokers, and the smoking group comprised both current and ex-smokers. Since most smokers had quit after their diagnosis of cancer, there were only 11 current smokers; therefore, we pooled current and ex-smokers in this study.

statistical methods

Data are presented as the median and range, unless stated otherwise. For statistical analysis, the χ^2 test for dichotomous variables or the Mann–Whitney U test for continuous variables was carried out. Logistic regression analysis was carried out to assess the correlation between gemcitabine-induced neutropenia and the following covariates: smoking history, age, gender, baseline neutrophil count, creatinine concentration, history of surgery for primary cancer, hemoglobin concentration, platelet count, aspartate aminotransferase (AST) concentration, alanine aminotransferase (ALT) concentration and total bilirubin concentration. $P < 0.05$ was regarded as significant. All statistical analyses were carried out using SAS version 9.1 (SAS Institute, Cary, NC).

results

patient characteristics

Data for 103 consecutive chemo-naive patients who underwent gemcitabine monotherapy from November 2003 to November 2007 at Kyoto University Hospital were analyzed. Patient baseline characteristics are summarized in Table 1. Fifty-nine patients had pancreatic cancer, 41 had hepatobiliary cancer, one had mesothelioma, one had liposarcoma and one had leiomyosarcoma. Fifty-one patients were classified into the smoking group and 52 patients into the non-smoking group. G-CSF was administered to three patients in the smoking group and eight in the non-smoking group. Baseline white blood cell (WBC) count, hemoglobin concentration and creatinine concentration were significantly higher in the smoking group, and the proportion of females was significantly higher in the non-smoking group. There was no significant difference between the numbers of patients in the smoking and non-smoking groups.

smoking history and gemcitabine-induced neutropenia

The overall incidence of grade 3–4 neutropenia was 24% in the smoking group and 56% in the non-smoking group [odds ratio (OR) 0.244, 95% confidence interval (CI) 0.105–0.569; $P < 0.001$; Table 2]. Logistic regression analysis was carried out using gender, age, baseline neutrophil count, creatinine concentration, history of surgery for primary cancer, hemoglobin concentration, platelet count, AST concentration, ALT concentration and total bilirubin concentration. A statistician confirmed the validity of the assumption of linearity by categorizing the continuous variables in the logistic regression analysis. After logistic regression analysis, a history of smoking was retained as an independent inverse predictor of gemcitabine-induced neutropenia (OR 0.188, 95% CI 0.057–0.618; $P = 0.006$; Table 3). The incidence of grade 3–4 neutropenia during the first cycle of gemcitabine treatment was higher in the non-smoking group than in the smoking group although the difference was not found to be significant in logistic regression analysis (OR 0.201, 95% CI 0.040–1.026; $P = 0.054$; Table 3).

discussion

Gemcitabine has a wide spectrum of antitumor activity with minimal non-hematological adverse events [1–4]. Although the recommended dosage regimen for gemcitabine monotherapy comprises three doses of 1000 mg/m^2 per cycle [1, 5, 6, 14], in the present study the maximum tolerated dose of gemcitabine (i.e. the dose that could be repeatedly administered without toxicity) ranged from 130 mg/m^2 to the recommended dose of 1000 mg/m^2 (Table 1). Thus, some patients required the gemcitabine dose to be reduced several times until a tolerable dose was reached. Actually, relative dose intensity of gemcitabine was significantly lower in the non-smoking group (Table 1), probably reflecting the fact that gemcitabine dose reduction was more common among non-smokers because of neutropenia.

Our analysis identified a significant inverse correlation between a history of smoking and the incidence of gemcitabine-induced neutropenia among chemo-naive patients treated with gemcitabine monotherapy (OR 0.244, 95% CI 0.105–0.569; $P < 0.001$; Table 2). There were only 11 current smokers in this study, two of whom developed grade 3–4 neutropenia, so to ensure robust statistical analysis we pooled the data from the ex-smokers and current smokers. Obviously, whether a patient is a current or an ex-smoker is likely to be pertinent, so future studies are warranted to evaluate the relative incidence of neutropenia among current smokers, ex-smokers and non-smokers. The proportion of women was significantly larger in the non-smoking group, which might have affected the current results; however, no clinically significant difference was found in gemcitabine clearance between men and women (E. Lilly, unpublished data). Smoking is known to increase the

Table 1. Patient characteristics

Characteristic	All patients (n =103)	Smoking group (n =51)	Non-smoking group (n =52)	P value
Gender				<0.01
Male	59 (57%)	43 (84%)	16 (31%)	
Female	44 (43%)	8 (16%)	36 (69%)	
Age (years)				0.63
Median	65	64	65	
Range	33–84	33–83	40–84	
Tumor type				0.75
Pancreatic	59 (57%)	30 (59%)	29 (56%)	
Hepatobiliary	41 (40%)	19 (37%)	22 (42%)	
Others	3 (3%)	2 (4%)	1 (2%)	
History of surgery for primary cancer	59 (57%)	27 (53%)	32 (62%)	0.38
Performance status (0/1/2)	(94/9/0)	(46/5/0)	(48/4/0)	
Total no. of gemcitabine administrations				0.69
Median	13	13	12	
Range	2–52	3–52	2–32	
Relative dose intensity of gemcitabine (mg/m^2)				0.03
Median	0.68	0.68	0.59	
Range	0.19–1.00	0.29–1.00	0.19–1.00	
Maximum tolerated dose of gemcitabine (mg/m^2)				0.13
Median	700	800	607	
Range	133–1000	200–1000	133–1000	
Baseline hematological data				
WBC count (× 10^9/l)				<0.01
Median	5.4	5.6	5.0	
Range	2.4–20.2	3–20.2	2.4–10.8	
Neutrophil count (× 10^9/l)				0.12
Median	3.3	3.4	2.9	
Range	1.7–17.2	3.2–17.2	1.1–8.8	
Hemoglobin concentration				<0.01
Median	12	12.3	11.5	
Range	7.2–17.9	8.8–17.9	7.2–15.5	
Platelet count (× 10^9/l)				0.48
Median	221	221	215	
Range	90–540	95–385	90–540	
Baseline blood chemistry				
AST concentration (IU/l)				0.90
Median	27	25	26	
Range	14–114	14–102	14–114	
ALT concentration (IU/l)				0.19
Median	30	31	26	
Range	9–167	9–167	10–125	
Total bilirubin concentration (mg/dl)				0.93
Median	0.7	0.7	0.7	
Range	0.3–4.0	0.4–4.0	0.3–2.6	
Creatinine concentration (mg/dl)				<0.01
Median	0.6	0.7	0.6	
Range	0.3–1.7	0.3–1.7	0.4–0.9	

The smoking group comprised patients who were current and ex-smokers. The non-smoking group comprised patients who had never been smokers. WBC, white blood cell; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

proliferation rate of myeloid progenitor cells [15–17], which might partly explain the significantly higher baseline WBC count and hemoglobin level in the smoking group in the present study (Table 1). The lower hemoglobin level in the non-smoking group could also be attributed to the larger proportion of women in this group. It is unlikely that G-CSF support affected our current results because G-CSF was used only after development of grade 3/4 neutropenia and was more common in the non-smoking group (three patients in the smoking group and eight patients in non-smoking group). Furthermore, G-CSF was never used to maintain gemcitabine dose intensity. We initially intended to examine the correlation between smoking history and gemcitabine-induced grade 3–4 anemia and thrombocytopenia; however, the incidences of grade 3–4 anemia and thrombocytopenia were too low (3.8% for both) to allow statistical analysis.

Logistic regression analysis with adjustment for age, gender, baseline neutrophil count, creatinine concentration, history of surgery for primary cancer, hemoglobin concentration, platelet count, AST concentration, ALT concentration and total bilirubin concentration identified smoking history as an independent predictive factor of gemcitabine-induced neutropenia (OR 0.188, 95% CI 0.057–0.618; $P = 0.006$; Table 3). Although the incidence of grade 3–4 neutropenia during the first cycle of treatment was higher in the non-smoking group than in the smoking group, the difference was not significant (OR 0.201, 95% CI 0.040–1.026; $P = 0.054$; Table 3). We speculate that this was due to the small sample size of the current study.

Interestingly, Laufman et al. [18] reported that smokers have a higher absolute neutrophil count than non-smokers when treated with gemcitabine monotherapy, while smokers have a lower absolute neutrophil count when treated with docetaxel monotherapy. These findings are consistent with our current results. Several mechanisms have been posited to explain the interaction between smoking habit and other antitumor drugs. Smoking potentially affects irinotecan metabolism, lowering exposure to the drug and reducing drug-induced neutropenia, at least in part by modulating CYP3A and UGT1A1 enzymes [9]. Smoking is also thought to enhance the clearance of erlotinib and lower the level of the drug in the blood by inducing CYP1A1/CYP1A2 enzyme expression [10, 11]. More than 90% of gemcitabine administered is converted into the inactive metabolite 2′-deoxy-2′,2′-difluorouridine by CDA [7]. Given that smoking is thought to modulate the irinotecan- and erlotinib-metabolizing enzymes as mentioned above, it is tempting to speculate that smoking could also affect gemcitabine metabolism by modulating CDA activity. There are other possible mechanisms. Several effects of smoking are known to persist for a long time after cessation of smoking. For example, the inflammatory response to smoking in patients with chronic obstructive pulmonary disease can continue after the patient stops smoking. Similarly, it can take at least 5 years for smoking-induced leukocytosis to resolve [17, 19, 20]. These reports prompted us to speculate that some unknown persistent change in ex-smokers also affected the current results. Further study is warranted to test this hypothesis.

In the non-smoking group, the incidence of gemcitabine-induced grade 3–4 neutropenia was 56%, which is much higher than previously reported figures, which have ranged from 20% to 30% [1, 6, 14]. In contrast, the corresponding incidence was 24% in the smoking group, which is comparable to previously reported values. Because of the high incidence of neutropenia (especially among non-smokers) and the broad range of

Table 2. Incidence of gemcitabine-induced grade 3–4 neutropenia according to smoking history

Time period	Smoking group ($n = 51$), n (%)	Non-smoking group ($n = 52$), n (%)	Odds ratio (95% CI)	P value
First cycle	4 (8)	19 (37)	0.148 (0.046–0.475)	0.001
Overall	12 (24)	29 (56)	0.244 (0.105–0.569)	<0.001

The smoking group comprised patients who were current and ex-smokers. The non-smoking group comprised patients who had never been smokers. CI, confidence interval.

Table 3. Odds ratios for the incidence of gemcitabine-induced grade 3–4 neutropenia during the first cycle and overall treatment

Factor	First cycle		Overall	
	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value
History of smoking	0.201 (0.040–1.026)	0.054	0.188 (0.057–0.618)	0.006
Gender	0.388 (0.074–2.027)	0.262	0.664 (0.168–2.614)	0.558
Age	0.992 (0.923–1.066)	0.825	0.939 (0.946–1.062)	0.939
Neutrophil count	0.320 (0.158–0.646)	0.001	0.500 (0.315–0.793)	0.003
Creatinine concentration	9.250 (0.281–304.505)	0.212	12.159 (0.585–252.754)	0.107
History of surgery for primary cancer	0.651 (0.165–2.580)	0.542	1.198 (0.402–3.568)	0.746
Hemoglobin concentration	0.869 (0.551–1.370)	0.545	1.231 (0.878–1.724)	0.228
Platelet count	0.998 (0.989–1.007)	0.720	1.002 (0.996–1.009)	0.476
AST concentration	1.049 (0.998–1.103)	0.061	1.057 (1.007–1.109)	0.024
ALT concentration	0.963 (0.916–1.011)	0.130	0.970 (0.939–1.002)	0.070
T-bilirubin concentration	0.561 (0.139–2.270)	0.418	0.525 (0.172–1.605)	0.259

CI, confidence interval; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

maximum tolerated doses, physicians sometimes start with a dose <1000 mg/m^2 to avoid neutropenia and repeated reduction in doses thereafter. But this approach comes with the risk of not achieving the maximum benefit from gemcitabine and should be avoided where possible; therefore, identifying patients at high risk of developing gemcitabine-induced neutropenia will help physicians to select an optimal gemcitabine dose.

Whether smoking also affects the antitumor effect of gemcitabine could not be investigated in the current study because of the heterogeneity of the study population with respect to tumor type and treatment aim (31 patients were treated with gemcitabine as adjuvant chemotherapy). Even if smoking does not affect the antitumor effect of gemcitabine, smoking remains highly detrimental to cancer patients in many ways and is clearly not an appropriate approach to avoid gemcitabine-induced neutropenia [21].

In summary, our present data indicate that patients without a history of smoking are at higher risk of developing grade 3–4 gemcitabine-induced neutropenia in daily clinical practice. Future studies including a larger number of patients are warranted to verify our results and to clarify the underlying mechanism.

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Prognostic value of metastin expression in human pancreatic cancer

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Abstract

Background: *KiSS-1* was identified as a metastasis-suppressing gene in melanoma cells. The *KiSS-1* gene product (metastin) was isolated from human placenta as the ligand of GPR54, a G-protein-coupled receptor. The role of metastin and GPR54 in tumor progression is not fully understood.

Methods: We investigated the clinical significance of metastin and GPR54 expression in pancreatic cancer. We evaluated immunohistochemical expression of metastin and GPR54 in pancreatic ductal adenocarcinoma tissues obtained from 53 consecutive patients who underwent resection between July 2003 and May 2007 at Kyoto University Hospital. In 23 consecutive patients, the plasma metastin level was measured before surgery by enzyme immunoassay.

Results: Strong immunohistochemical expression of metastin was detected in 13 tumors (24.5%), while strong expression of GPR54 was detected in 30 tumors (56.6%). Tumors that were negative for both metastin and GPR54 expression were significantly larger than tumors that were positive for either metastin or GPR54 ($p = 0.047$). Recurrence was less frequent in patients who had metastin-positive tumors compared with those who had metastin-negative tumors (38.5% versus 70.0%, $p = 0.04$). Strong expression of metastin and GPR54 was significantly correlated with longer survival ($p = 0.02$). Metastin expression by pancreatic cancer was an independent prognostic factor for longer survival (hazard ratio, 2.1; 95% confidence interval, 1.1–4.7; $p = 0.03$), and the patients with a high plasma metastin level ($n = 6$) did not die after surgical resection.

Conclusion: Strong expression of metastin and GPR54 by pancreatic cancer is associated with longer survival. Metastin expression is an independent prognostic factor for the survival of pancreatic cancer patients. The plasma metastin level could become a noninvasive prognostic factor for the assessment of pancreatic cancer.

Background

Pancreatic cancer remains a lethal disease and is the fourth to fifth leading cause of cancer-related death in the Western world, despite a significant reduction of the postoperative morbidity and mortality associated with pancreatectomy[1,2]. While surgical resection represents the only definitive option for cure of this disease and complete tumor resection is associated with longer survival, only 10% to 15% of patients have resectable disease[3,4]. Most patients with pancreatic cancer have locally advanced tumors, metastases, or both at the time of diagnosis. In addition, tumors frequently recur, even after margin-free curative resection, and most patients with recurrence have metastasis, which is often fatal. To improve the survival of patients with pancreatic cancer, we need a new strategy for the treatment of advanced disease that is unsuitable for surgical resection.

Metastasis is a multistep process in which tumor cells migrate through the stroma and invade a vessel, after which the cells are transported through the circulation to re-invade and proliferate at a distant site. Dozens of regulators influence each step of the metastatic cascade[5,6]. In 1996, *KiSS-1* was identified as a human metastasis-suppressing gene in melanoma cells[7] and breast cancer cells[8]. Then, the *KiSS-1* gene product was isolated from human placenta as the endogenous ligand of an orphan G-protein-coupled receptor known as GPR54[9], AXOR12[10], or hOT7T175[11]. *KiSS-1* encodes a 145-amino acid peptide which is further processed to a C-terminally amidated peptide with 54 amino acids called metastin[11] or kisspeptin-54, as well as to peptides with 14 amino acids (kisspeptin-14) and 13 amino acids (kisspeptin-13)[9].

The bioactive sequence of the *KiSS-1* gene product is the C-terminal 10 amino acids, metastin (45–54) (metastin-10 or kisspeptin-10)[12]. Metastin was shown to inhibit the chemotaxis and invasion of *GPR54*-transfected Chinese hamster ovary cells *in vitro*, while it inhibited the pulmonary metastasis of *GPR54*-transfected melanoma cells *in vivo*[11]. The prognostic relevance of *KiSS-1* has been demonstrated for some solid tumors [13-21].

In addition to the inhibition of tumor metastasis, *KiSS-1* shows neuroendocrine activity and has a role in the gonadotropin-releasing hormone cascade, puberty, placentation, and reproduction, as shown by recent studies[22,23]. In normal tissues, the highest level of *KiSS-1* mRNA expression has been detected in the placenta, with moderate to weak expression in the central nervous system, testis, liver, pancreas, and intestine[7,10,11]. In the case of *GPR54* mRNA, high levels of expression are found in the placenta, pancreas, and central nervous system [9-11].

We previously found that expression of *KiSS-1* mRNA was lower and expression of *GPR54* mRNA was higher in pancreatic cancer tissue compared with normal pancreatic tissue[24]. However, the clinical significance of *KiSS-1* and *GPR54* expression by pancreatic cancer remains unclear. We hypothesized high levels of *KiSS-1* and *GPR54* expression could be associated with better survival of pancreatic cancer patients. Therefore, we investigated immunohistochemical expression of the *KiSS-1* gene product (metastin) and that of GPR54 in pancreatic cancer tissues obtained by surgical resection. We also measured plasma metastin levels in pancreatic cancer patients by using an enzyme immunoassay (EIA) that we previously established[25] and evaluated the clinical applicability of these two parameters.

Methods

Patients

A total of 53 consecutive patients with pancreatic cancer who underwent surgical resection between July 2003 and May 2007 at Kyoto University Hospital were studied. The diagnosis of ductal adenocarcinoma of the pancreas was confirmed histologically by at least two pathologists who examined the resected specimens. None of the patients received preoperative chemotherapy or radiation therapy, and all patients gave written informed consent to participation in the study. Follow-up information was obtained from the medical records or by direct contact with patients or their referring physicians.

We evaluated the following clinicopathological characteristics according to the sixth edition of the TNM classification of the international union against cancer (UICC)[26]: tumor location, tumor size, tumor extent (pT), lymph node metastasis (pN), pStage, histopathological grade (G), lymphatic invasion, venous invasion, perineural invasion, and residual tumor (R).

Immunohistochemical staining for metastin and GPR54

Immunohistochemical staining of resected pancreatic tissues was done in 53 patients with ductal adenocarcinoma of the pancreas. We chose sections that contained cancer tissue and adjacent non-cancerous tissue in the same section.

Paraffin-embedded tissue blocks were cut into 4 μm sections, dried overnight at 37°C, and then deparaffinized with xylene and rehydrated in a graded ethanol series. Sections were treated with Dako target retrieval solution (Dako, Carpinteria, CA, USA) before antigen retrieval was done by heating at 95°C for 40 min. Then the sections were cooled to room temperature, and were treated with dilute hydrogen peroxide to block endogenous peroxidase activity. Nonspecific binding was minimized by incubation with Dako protein block (Dako) for 30 min. Rabbit

anti-human polyclonal antibodies for metastin (1–54)-Amide (catalogue number: H-048-59, Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA) and GPR54 (375–398) (catalogue number: H-048-61, Phoenix Pharmaceuticals) were applied overnight at 4°C at a dilution of 1:400. On the next day, sections were incubated for 1 hr at room temperature with anti-rabbit IgG conjugated to a horseradish peroxidase (HRP) -labelled polymer (Dako Envision™ + System, Dako), treated with 3,3'-diaminobenzidine tetrahydrochloride (DAB), and counterstained with Mayer's hematoxylin. As a positive control, human placental tissue was stained with the anti-metastin and anti-GPR54 antibodies (Figure 1A, 1B). For negative control slides, the primary antibody was substituted with irrelevant rabbit serum.

Assessment of metastin and GPR54 expression

Five fields (at a × 400 magnification) were randomly chosen to evaluate staining. The intensity of staining in cancer tissues was graded according to a 3-point scale as follows: 0 was weak; 1 was mild (the same staining intensity as that of non-cancerous pancreatic ducts as an internal control on each slide); and 2 was strong. The percentage of tumor cells showing each staining intensity was estimated to calculate an intensity score ([0 × %weak] + [1 × %mild] + [2 × %strong]) that could range from 0 to 200. A score ≥ 100 was defined as positive staining and a score <100 was defined as negative staining.

Then we compared clinicopathological characteristics between patients with positive and negative staining for metastin and GPR54.

Blood sampling and EIA for plasma metastin

Plasma levels of metastin were measured by EIA, as described previously[25], in 23 consecutive patients who underwent resection between July 2006 and May 2007.

A blood sample was collected in the morning before surgery, placed in a chilled tube containing aprotinin (500 KIU/ml) and EDTA (1.2 mg/ml), and immediately centrifuged. The plasma thus obtained was diluted five-fold with 4% acetic acid (pH 4.0), and loaded onto a column with a C18 reversed-phase cartridge (Sep-Pak C18, Millipore, Milford, MA, USA). After washing with 4% acetic acid, peptides were eluted with 70% acetonitrile in 0.5% acetic acid (pH 4.0). The eluted samples were concentrated by spin-vacuum evaporation, lyophilized, and stored at -40°C until assay.

EIA was performed by the delayed-addition method with separation of bound and free antigens on anti-rabbit IgG-coated immunoplates. Human metastin (45–54) was conjugated with β-D-galactosidase using *N*-(ε-maleimidocaproyloxy)-succinimide, as reported previously[27]. The EIA was sensitive and specific for all bioactive *KiSS-1* gene products (metastin, kisspeptin-14, and kisspeptin-13)[25].

The third quartile value was set as a cut-off for the plasma metastin level. We evaluated the association between the plasma level of metastin and metastin immunoreactivity in resected pancreatic cancer tissues, and also the associations between plasma metastin and the clinicopathological characteristics of the patients.

Statistical analysis

Continuous variables are presented as the mean ± standard deviation or as the median and range. Comparison of the groups was done with the Mann-Whitney U test, while categorical variables were compared by the χ^2 test. Correlations between metastin and GPR54 immunoreactivity were investigated by calculation of Pearson's correlation coefficient (r) values and scatter plots with a linear regression line were drawn. An r value of 0–0.19 was defined as a very weak correlation, while 0.2–0.39 was weak, 0.40–0.59 was moderate, 0.6–0.79 was strong, and 0.8–1 was very strong. Overall survival curves were drawn by the Kaplan-Meier method, and were compared by the log-rank test. Prognostic factors for survival were examined by univariate and multivariate analyses using Cox's proportional hazards model. For all analyses, $p < 0.05$ was considered to be statistically significant.

Results

Demographic and clinicopathological characteristics

There were 25 men (47.2%) and 28 women (52.8%) with a mean age at diagnosis of 65.6 years (median age: 68 years; range: 32 - 86 years). The tumor was located in the head of the pancreas in 38 patients (71.7%), while it was found in the distal pancreas in 15 patients (28.3%). Pancreatoduodenectomy was performed in 36 patients (67.9%), while distal pancreatectomy was performed in 13 patients (24.5%), and total pancreatectomy in 4 patients (7.5%). On histopathological examination, one patient (1.9%) had pStage IA disease, three patients (5.7%) had pStage IB, 16 patients (30.2%) had pStage IIA, 29 patients (54.7%) had pStage IIB, and four patients (7.5%) had pStage IV.

Twenty-nine patients received adjuvant chemotherapy, which consisted of S-1 (n= 18), gemcitabine (n = 8), 5-fluorouracil (n = 2), and tegafur-uracil (n = 1). This was excluded from statistical analysis because of variations in the duration and type of chemotherapy.

Immunostaining for metastin and GPR54

Pancreatic cancer tissues showed heterogenous immunoreactivity for metastin and GPR54 (Figure 1). Acinar cells and islet cells did not exhibit any immunoreactivity, while

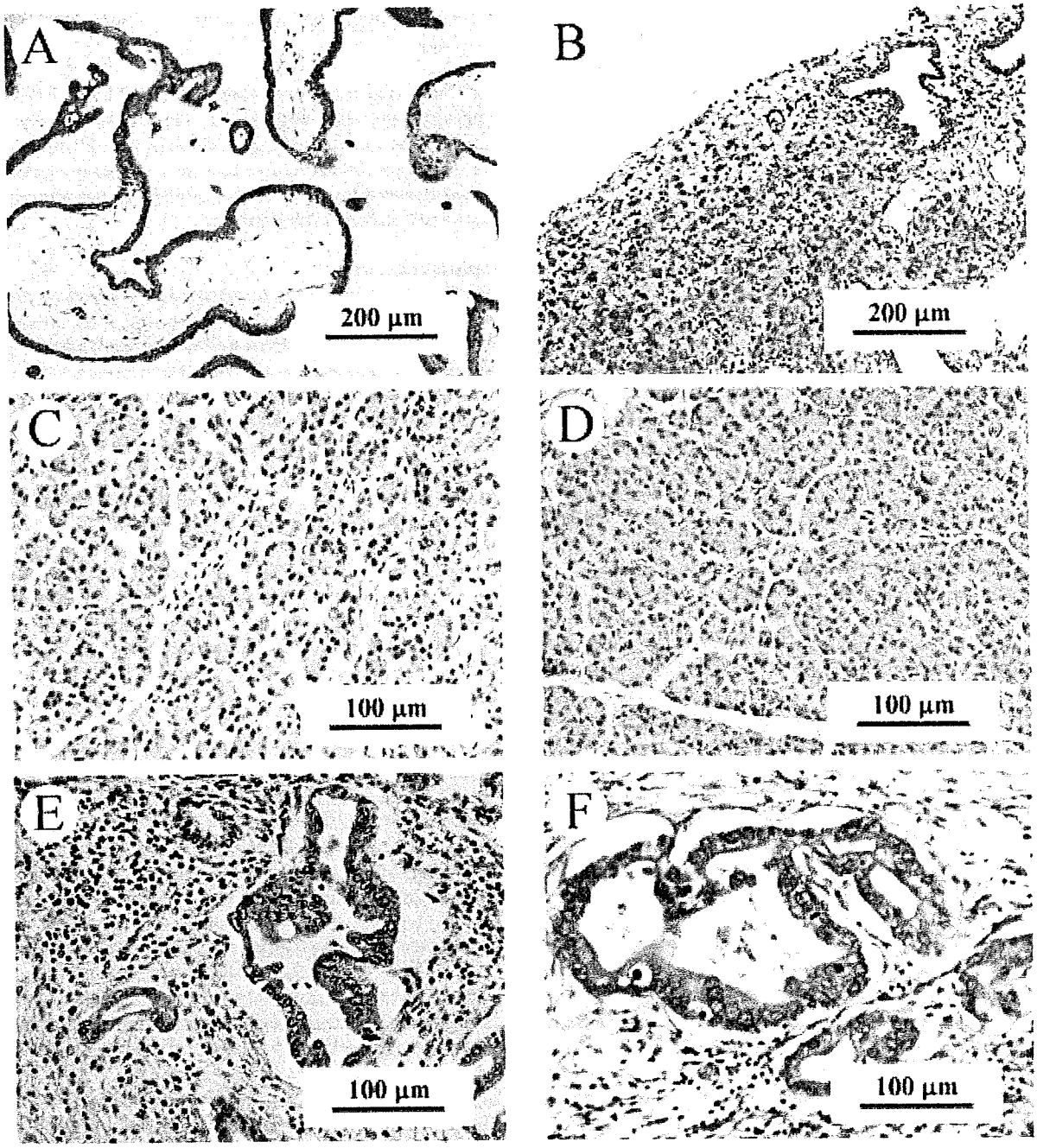

Figure 1
Immunohistochemical staining of non-cancerous pancreatic tissues and pancreatic cancer tissues. (A, B); Immunohistochemical staining of human placental tissues as a positive control. Tissues were stained with anti-metastin (A) and anti-GPR54 antibody (B). (Original magnification, × 200). (C, D); Non-cancerous and cancerous tissues were stained with anti-metastin and anti-GPR54 antibody. (Original magnification, × 400). Weak positivity of non-cancerous ductal cells for metastin (C) and GPR54 (D). (E, F); Pancreatic cancer tissues were stained with anti-metastin and anti-GPR54 antibody. Heterogeneous strong positive immunostaining of carcinoma cells for metastin (E) and GPR54 (F) are shown.

metastin and GPR54 were both weak or mildly positive in the cytoplasm of normal pancreatic ductal cells.

The mean intensity score for metastin was 72.1 ± 54.9 (n = 53) and that for GPR54 was 99.9 ± 55.1 (n = 53) (Figure 2).

Positive metastin staining was detected in 13 tumors (24.5%), while GPR54 was positive in 30 tumors (56.6%). Immunoreactivity for metastin and GPR54 showed a strong positive correlation (r = 0.62, p < 0.001; Fig. 3).

Demographic and clinicopathological characteristics showed no significant differences between patients whose tumors were positive or negative for metastin (Table 1), and the outcome was similar for GPR54 (Table 2). However, tumors that were negative for both metastin and GPR54 showed a significantly larger size than tumors positive for metastin and/or GPR54 (median of 2.5 cm and range of 0.8–5.0 cm versus median of 3.0 cm and range of 1.5–6.5 cm, p = 0.047).

Recurrence and survival

The median postoperative follow-up period was 18.5 months (range: 2.6–59.2 months). There were no operative deaths in this series. During the follow-up period, 33 patients (62.3%) showed recurrence and 25 patients (47.2%) died of their cancer. Recurrence was detected in the liver (n = 15), local region (n = 9), peritoneum (n = 9), lymph nodes (n = 5), lungs (n = 1), and bone (n = 1), while it was at an unknown location in 1 patient (elevated tumor marker). No patient died of any other disease or cause.

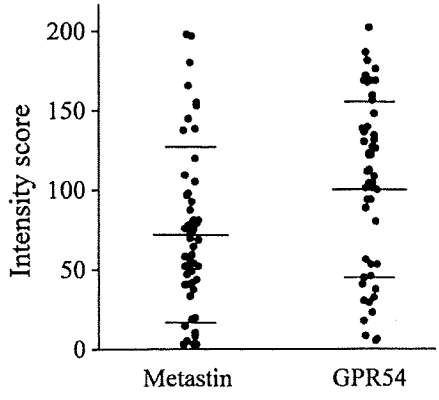

Figure 2
Expression of metastin and GPR54 in pancreatic cancer tissues. Immunoreactivity for metastin and GPR54 in resected pancreatic cancer tissues (n = 53) shown as the intensity score of each patient. The mean metastin intensity score was 72.1 ± 54.9 and that for GPR54 was 99.9 ± 55.1. The horizontal bar indicates the mean ± SD.

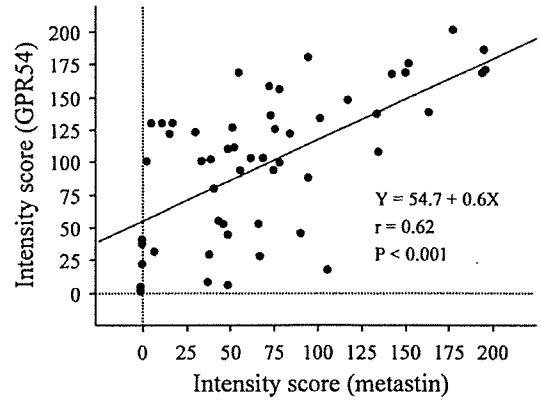

Figure 3
Correlation between metastin and GPR54 expression in pancreatic cancer tissues. Scatter plot showing the correlation between immunoreactivity for metastin and GPR54. A strong correlation was found (r = 0.62, p < 0.001).

The recurrence rate was significantly lower in the patients whose tumors were positive for metastin than in those with negative tumors (38.5% versus 70.0%, p = 0.04) (Table 3). There were no significant differences of the recurrence rate at each site between the patients with metastin-positive and -negative tumors (Table 3), and the same was found for GPR54 (Table 4).

The overall survival of patients whose tumors were positive for metastin was significantly longer than that of patients with negative tumors (p = 0.02) (Figure 4). Similarly, the overall survival of patients with tumors that were positive for GPR54 was significantly longer than that of patients with negative tumors (p = 0.02) (Figure 5).

Prognostic factors according to multivariate analysis

Univariate and multivariate analysis were performed to identify parameters associated with overall survival according to the Cox proportional hazards model. The univariate analysis revealed the following five factors to be associated with survival: perineural invasion, pStage, residual tumor, metastin expression, and GPR54 expression. In the multivariate analysis, as well as the UICC pStage (I + II versus IV), overexpression of metastin was an independent prognostic factor for better survival (hazard ratio, 2.08; 95% confidence interval, 1.05–4.71; p = 0.03) (Table 5).

Table 1: Comparison of the patients with pancreatic cancer who had positive immunostaining for metastin and those negative.

Characteristics	Positive for metastin (n = 13)	Negative for metastin (n = 40)	P value
Age	68.8 ± 7.2 (71, 56–78)	64.5 ± 10.5 (65.5, 32–86)	0.19
Gender			
Male	6	19	0.93
Female	7	21	
Location of tumor			
Pancreas head	8	30	0.35
Pancreas body-tail	5	10	
Size of tumor, cm	2.5 ± 0.9 (2.5, 1.2–4.5)	3.0 ± 1.2 (2.8, 0.8–6.5)	0.34
Histopathological grading			
G1	5	9	0.26
G2-4	8	31	
pT			
pT1, pT2	2	6	0.97
pT3	11	34	
pN			
pN0	6	15	0.58
pN1	7	25	
Lymphatic invasion			
Positive	7	24	0.70
Negative	6	16	
Venous invasion			
Positive	7	23	0.82
Negative	6	17	
Perineural invasion			
Positive	6	22	0.58
Negative	7	18	
pStage			
I, II	13	36	0.24
IV	0	4	
Residual tumor			
R0	11	28	0.30
R1	2	12	

Median and range are shown in parentheses.

Plasma metastin level

The mean plasma level of metastin before surgery was 22.7 ± 17.2 fmol/ml (median, 21.5 fmol/ml; range, 4.0–58.9 fmol/ml). Plasma metastin levels and the intensity score for metastin immunoreactivity in resected tissues showed a weak correlation (r = 0.23, p = 0.30). When we used the third quartile plasma metastin level (28.0 fmol/ml) as a cut-off value, there were no significant differences of demographics and clinicopathological characteristics between patients with a high (n = 6) or low (n = 17) plasma metastin level.

Overall survival curves of the patients with high and low plasma metastin levels are shown in Fig. 6. The median postoperative follow-up period was 14.8 months (range: 2.6–22.1 months, n = 23). While survival showed no significant difference between the two groups (p = 0.14), no patient with a high plasma metastin levels died after surgery (Figure 6).

Discussion

In this study, we investigated the clinical significance of immunohistochemical metastin and GPR54 expression in resected pancreatic cancer tissues. We found that strong expression of metastin or GPR54 was associated with better survival, and metastin expression was an independent prognostic factor for longer survival of pancreatic cancer patients. Our results indicate that the metastin/GPR54 signaling system acts to suppress the growth of pancreatic cancer.

Recently, the prognostic relevance of *KiSS-1* and *GPR54* has been investigated in some solid tumors [13-21]. Most of these studies have shown that the *KiSS-1/GPR54* system is negatively correlated with tumor progression. *KiSS-1* has been demonstrated to act as a suppressor in melanoma[13], thyroid cancer[14], bladder cancer[16], gastric cancer[17], esophageal cancer[18], and ovarian cancer[20].

Table 2: Comparison of the patients with pancreatic cancer who had positive immunostaining for GPR54 and those negative.

Characteristics	Positive for GPR54 (n = 30)	Negative for GPR54 (n = 23)	P value
Age	66.1 ± 8.7 (65.5, 49–86)	64.9 ± 11.5 (68.0, 32–80)	0.99
Gender			
Male	12	13	0.23
Female	18	10	
Location of tumor			
Pancreas head	21	17	0.75
Pancreas body-tail	9	6	
Size of tumor, cm	2.7 ± 1.0 (2.5, 0.8–5.0)	3.1 ± 1.2 (3.0, 1.2–6.5)	0.13
Histolopathological grading			
G1	10	4	0.19
G2-4	20	19	
pT			
pT1, pT2	6	2	0.25
pT3	24	21	
pN			
pN0	13	8	0.53
pN1	17	15	
Lymphatic invasion			
Positive	18	13	0.80
Negative	12	10	
Venous invasion			
Positive	18	12	0.57
Negative	12	11	
Perineural invasion			
Positive	15	13	0.64
Negative	15	10	
pStage			
I, II	29	20	0.18
IV	1	3	
Residual tumor			
R0	24	15	0.23
R1	6	8	

Median and range are shown in parentheses.

For example, Shirasaki et al[13] showed that downregulation of *KiSS-1* is important for the progression of melanoma in vivo. Ringel et al[14] showed that *KiSS-1* and *GPR54* mRNA were overexpressed in papillary thyroid cancer compared with follicular cancer. In bladder cancer, loss of *KiSS-1* expression is related to tumor progression[16]. In gastric cancer, lower expression of *KiSS-1* mRNA is associated with venous invasion, distant metastasis, and tumor recurrence[17]. Furthermore, *KiSS-1* is an independent prognostic marker for gastric cancer according to multivariate analysis [17]. Ikeguchi et al. [18] observed that loss of *KiSS-1* mRNA, *GPR54* mRNA, or

Table 3: The rate and site of recurrence after resection of pancreatic cancer in relation to metastin expression.

	Metastin expression Positive (n = 13)	Metastin expression Negative (n = 40)	P value
Recurrence, n (%)	5 (38.5%)	28 (70.0%)	**0.04**
Site of recurrence			
Liver, n (%)	4 (30.8%)	11 (27.5%)	0.82
Local, n (%)	2 (15.4%)	7 (17.5%)	0.86
Peritoneum, n (%)	1 (7.7%)	8 (20.0%)	0.30
Lymph nodes, n (%)	1 (7.7%)	4 (10.0%)	0.80
Lungs, n (%)	0	1 (2.5%)	0.56
Bone, n (%)	0	1 (2.5%)	0.56
Unknown*, n (%)	0	1 (2.5%)	0.56

* Confirmed by elevated tumor marker during follow-up