Table 1. Characteristics of the patients

	Conventional clamp group (n = 40)	Drain clamp group (n = 47)	χ^2	p value
Gender (M/F)	28/12	25/22	2.56	0.11
Age, years				
Mean ± SEM	61.7 ± 1.7	65.4 ± 1.2	2.25	0.13
Range (median)	43 – 81 (64)	47 – 81 (66)		
Benign tumor/malignant tumor	6/34	6/41	0.09	0.76
Pancreatic ductal cancer	21	23		
Pancreatic cystic neoplasm	7	6		
Bile duct cancer	4	6		
Ampullary cancer	3	6		
Chronic pancreatitis	2	3		
Endocrine tumor	2	0		
Metastatic renal cancer	1	1		
Malignant lymphoma	0	1		
Duodenal cancer	0	1		
PD/PPPD	21/19	19/28	1.27	0.26

hole placed in the middle part of the clamp. When common hepatic duct was divided, a 12- or 14-Fr Safeed™ nelaton catheter (Terumo Corporation, Tokyo, Japan) was inserted into the hepatic duct stump, and the drainage clamp was placed to bite the common duct together with the nelaton catheter fixed (fig. 2). After removal of the duodenum and the pancreatic head, reconstruction was made by end-to-side pancreaticojejunostomy, end-to-side hepaticojejunostomy and end-to-side gastro- or duodenojejunostomy in this order (modified Child's method) in both groups. In all patients, biliary stent tube was not placed.

Laboratory and Clinical Data

Preoperative, perioperative, and postoperative laboratory and clinical data were retrospectively collected by chart review. As a marker for liver function and systemic inflammation, we have collected the values of total bilirubin, AST, ALT, white blood cell counts and C-reactive protein just before operation and in the first 14 postoperative days.

Major complications recorded in the postoperative period included postoperative death (death during the hospital stay for surgery or within 30 days of surgery); reoperation (during the hospital stay for surgery); postoperative intra-abdominal bleeding; intra-abdominal abscess; increased amylase in drain (drain amylase level more than 5,000 IU/l on any postoperative day without clinical sequelae); pancreaticojejunal anastomotic leak (drain amylase level more than 5,000 IU/l on any postoperative day with clinical sequelae such as fever, leukocytosis, fistula, or abscess); other anastomotic leaks (from the hepaticojejunal, gastrojejunal or duodenojejunal anastomosis); sepsis syndrome; pneumonia; gastrointestinal bleeding; and pulmonary embolism.

Other complications recorded in the postoperative period included allergic reaction, atelectasis (radiographic or clinical), cardiac arrhythmia, wound infection, cholangiitis, pancreatitis, delayed gastric emptying (gastrostomy tube output >1,000 ml on postoperative day 7 or inability to tolerate a postgastrectomy diet by postoperative day 10), ileus (absence of flatus and/or bowel sounds beyond postoperative day 7), infectious colitis (as documented by *Clostridium difficile* toxin assay), urinary tract infection (documented by positive urine culture), deep vein thrombosis, chylous ascites, pleural effusion (radiographic or clinical) and liver dysfunction (defined as either a peak AST or a peak ALT >500 IU/l).

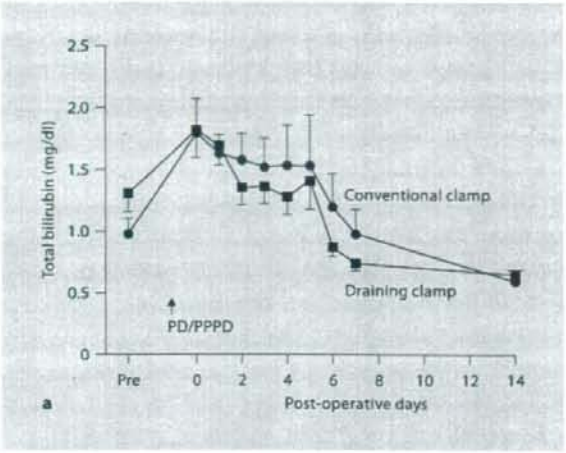
Statistical Analysis

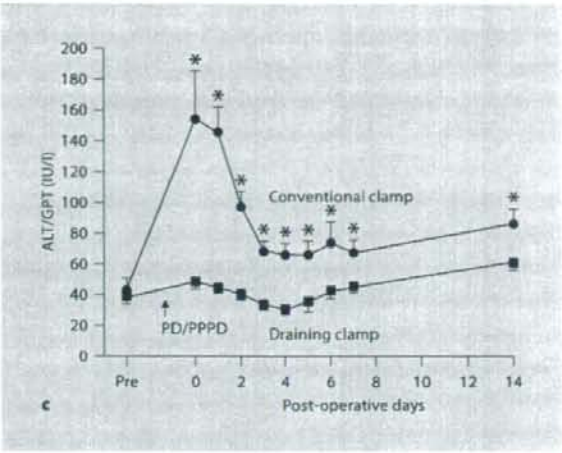
Results are expressed as the means and SEMs. Patient characteristics and perioperative and postoperative factors between 2 groups were compared by Mantel-Haenszel test. Distributions of numeric variables between groups were compared by analysis of variance, followed by a post hoc Tukey-Kramer test when appropriate. $p < 0.05$ was considered statistically significant.

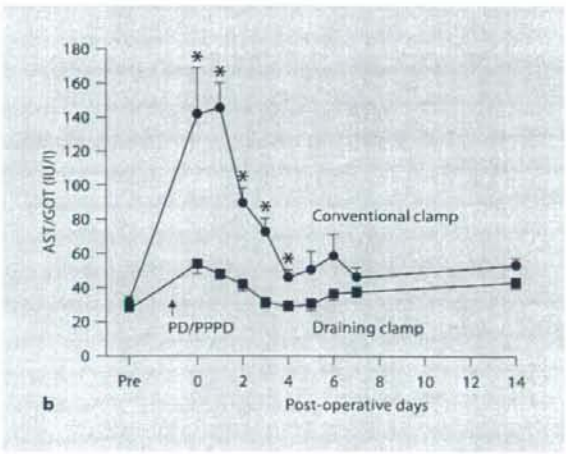
Results

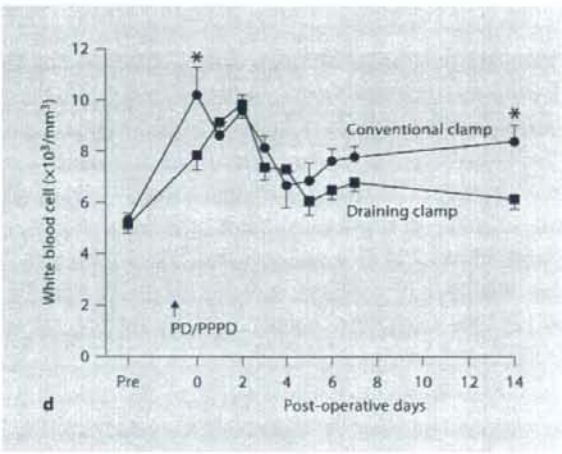
A total of 87 patients who underwent pancreaticoduodenectomy were included; the conventional clamp group consisted of consecutive 40 patients and the novel drainage clamp group consisted of consecutive 47 patients. The clinical characteristics of these 87 patients are summarized in table 1. These two patient groups are well matched for age, gender, operative time, intraoperative blood loss, transfusion requirements, pathology, and type of resection. Their preoperative liver enzyme profiles were also similar.

Postoperative liver function was assessed by the total bilirubin, AST, and ALT levels for 14 PODs (fig. 3). The total bilirubin levels of the conventional clamp group and drainage clamp group were 1.6 ± 0.2 and 1.7 ± 0.2 mg/dl, respectively, at 6 h after operation (POD 0) which was the peak value for both groups during the tested period,









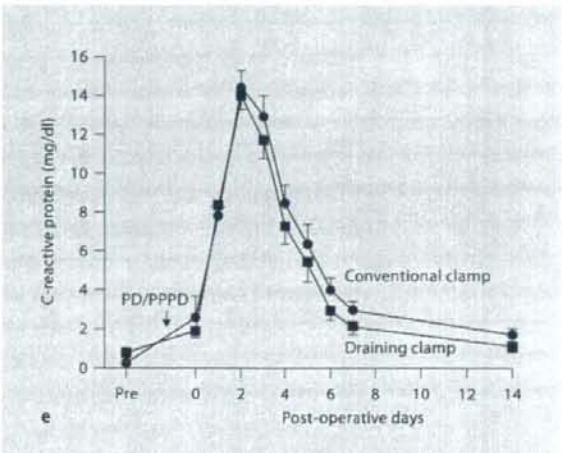


Fig. 3. Changes of total bilirubin levels (**a**), AST levels (**b**), ALT levels (**c**), WBC counts (**d**), and CRP levels (**e**) after pancreatoduodenectomy. * Significant difference between groups.

Table 2. Postoperative complications

	Conventional clamp group (n = 40)	Drain clamp group (n = 47)	p value
Death	0 (0)	0 (0)	n.a.
Reoperation	0 (0)	0 (0)	n.a.
Intra-abdominal bleeding	0 (0)	0 (0)	n.a.
Abdominal abscess	1 (3)	0 (0)	0.28
Increase of amylase in drain fluid	2 (5)	2 (4)	0.87
Pancreaticojejunal anastomotic leak	1 (3)	0 (0)	0.28
Bile leakage from the hepaticojejunostomy	0 (0)	0 (0)	n.a.
Leakage from the gastro(duodeno)-jejunostomy	0 (0)	0 (0)	n.a.
Gastrointestinal bleeding	1 (3)	0 (0)	0.28
Wound infection	2 (5)	2 (4)	0.87
Acute pancreatitis	0 (0)	0 (0)	n.a.
Delayed gastric emptying	2 (5)	1 (2)	0.47
Increase of transaminases	32 (80)	9 (19)	<0.001

n.a. = Not applicable. Data are the number (%) of patients.

and the bilirubin levels decreased gradually (fig. 3a). The postoperative total bilirubin levels did not differ between the two groups.

The AST level of the conventional clamp group hit the peak at approximately 12 h after surgery on the POD 1, and decreased gradually; however, the levels were significantly higher than those of drainage clamp group until the POD 4 (fig. 3b). After the POD 4, the AST level was not statistically different, but that of conventional clamp group was slightly higher than that of drainage clamp group.

The ALT level of the conventional clamp group hit the peak at 6 h after surgery (on the POD 0), and decreased gradually; however, the levels were significantly higher than those of drainage clamp group until the POD 14 (fig. 3c).

Postoperative inflammatory response was assessed by the peripheral white blood cell (WBC) count and serum C-reactive protein (CRP) levels for 14 PODs (fig. 3d, e). The WBC count was not much different between the two groups though, at 6 h after operation and on POD 14, the WBC count of the conventional clamp group was significantly higher than that of the drainage clamp group (fig. 3d). The CRP levels increased postoperatively and hit the peak on the POD 2 in the two groups (fig. 3e). The postoperative CRP levels did not differ between the two groups.

There was no operative death in both groups; further, as listed in table 2, there was no major leakage of the pancreaticojejunostomy or intra-abdominal bleeding. The rate of other postoperative complications was comparable between the two groups except for liver dysfunction defined by the increased AST/ALT. Increased AST/ALT was observed 32 of the 40 patients with conventional clamp (80%) and 9 of the 47 patients with drainage clamp (19%) ($p < 0.001$).

Discussion

During the postoperative period, many patients who undergo pancreatoduodenectomy have elevated serum liver enzymes of varying degrees. In most patients, this biochemical abnormality is temporary and the serum levels gradually return to normal; however, minimizing intraoperative liver damage is important after such a major operation because the liver plays a key role in recovery from the surgical trauma. First, the liver forms and secretes albumin, procoagulant factors, and acute phase reactant proteins; second, it metabolizes waste, drugs, and toxins; and third, it plays a key role in immunological response. Therefore, there is a strong possibility that postoperative liver dysfunction increases the incidence of, and compromises recovery from, other possible complications.

Intraoperative biliary decompression after dissection of the common hepatic duct by a retrograde transhepatic biliary catheter has been shown to reduce the postoperative transaminase levels within the first 7 PODs [4]. In addition, the number of patients with postoperative in-

creases of transaminase level higher than 500 IU/l was significantly less in the biliary decompression group than in the group without decompression. In agreement with this study, the results of the current study showed that intraoperative closure of the common hepatic duct resulted in elevated postoperative transaminase levels, and intraoperative drainage by our novel method significantly reduced the transaminase levels to almost normal range. The number of patients with postoperative increase of transaminase levels was significantly less in the intraoperative drainage group than in the group without drainage. These results suggest that the postoperative liver dysfunction observed after pancreatoduodenectomy is, at least, partially due to intraoperative prolonged closure of the common hepatic duct in most cases. Furthermore, intraoperative drainage by our novel drainage clamp can reduce intraoperative liver damage and prevent postoperative liver dysfunction.

When a PTBD catheter is placed in patients with jaundice preoperatively, the catheter is left, and can be used for the purpose of decompression in the hepatic duct during the postoperative period. However, it has been reported that many centers perform surgery without biliary drainage even in jaundiced patients [2, 3]. Therefore, if a PTBD catheter is not placed preoperatively, which may be the common status in patients who are scheduled to undergo pancreatoduodenectomy, the intrahepatic biliary pressure will be elevated when the common hepatic duct is dissected and closed with a conventional clamp during operation. In addition, frequent and intermittent bile drainage by opening the conventional clamp during operation would be time-consuming and might increase the possibility of postoperative infective complications by the contaminated bile.

Intraoperative insertion of a retrograde transhepatic biliary drainage catheter has been proposed as a solution to decrease biliary pressure [4]; however, placement of this catheter has been reported to accompany several complications such as local peritonitis [5], biliary stricture [6] and intrahepatic arterial bleeding. In contrast, the present method of intraoperative biliary drainage is simple and safe; the operator just needs to insert a soft silicon tube into the hepatic duct and place the novel clamp on the hepatic duct. It requires only 1 min without contamination and danger. We have not experienced any adjacent tissue injury or organ injury by this new clamp system, and intra-abdominal abscess formation postoperatively.

In conclusion, we demonstrated that continuous decompression of the hepatic duct during pancreatoduodenectomy is beneficial to patients by avoiding liver dysfunction. The novel drainage clamp, which facilitates intraoperative hepatic duct drainage, is a safe and useful tool for pancreatoduodenectomy and other operative procedure where extrahepatic bile duct is dissected.

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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

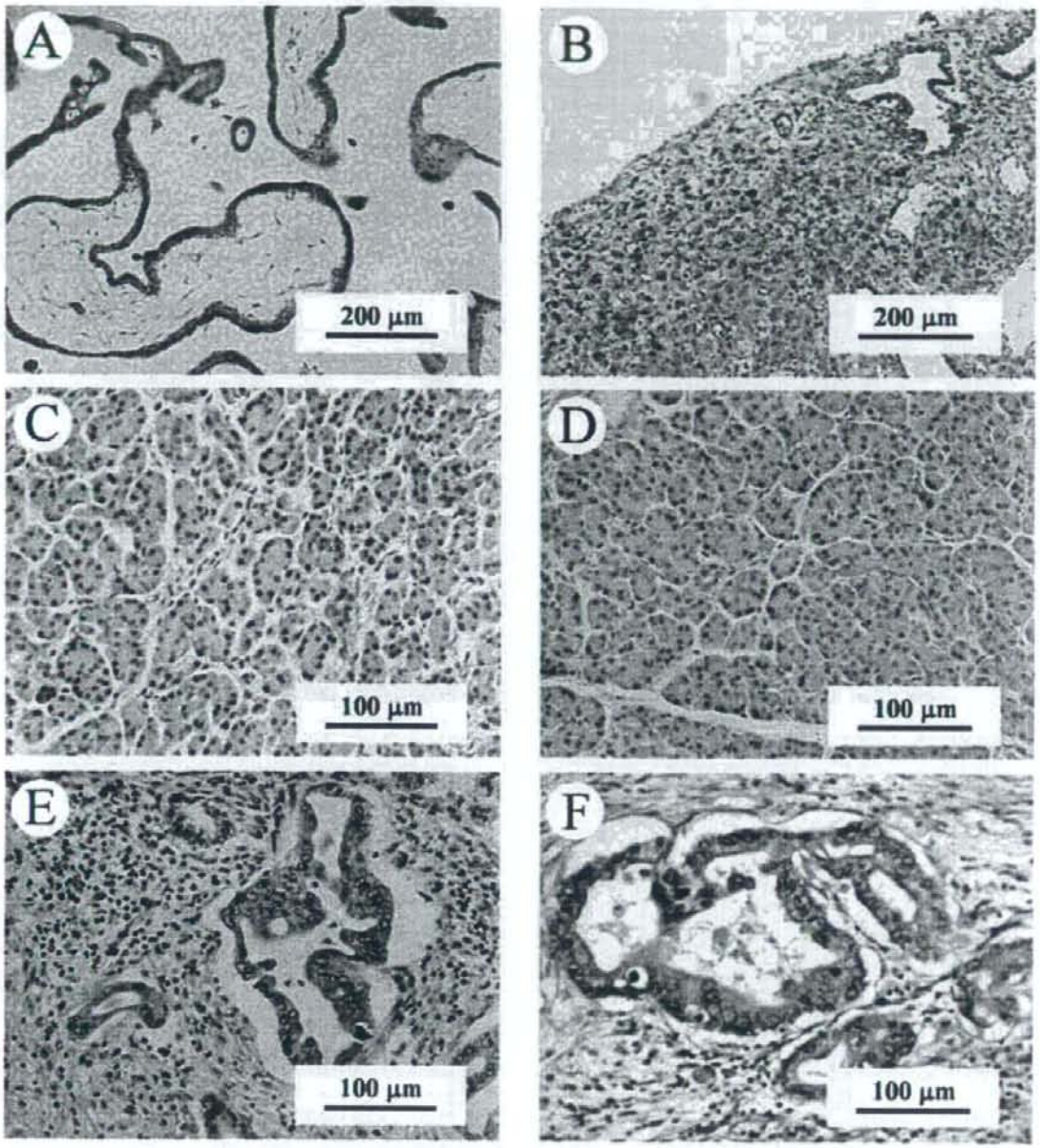

Figure 1

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.

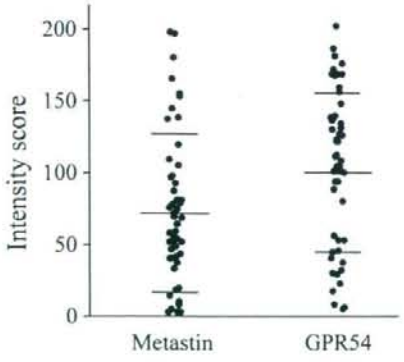

Figure 2
Expression of metastin and GPR54 in pancreatic cancer tissues. Immunoreactivity for metastin and GPR54 in resected pancreatic cancer tissues (n = 53) shown as the intensity score of each patient. The mean metastin intensity score was 72.1 ± 54.9 and that for GPR54 was 99.9 ± 55.1. The horizontal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

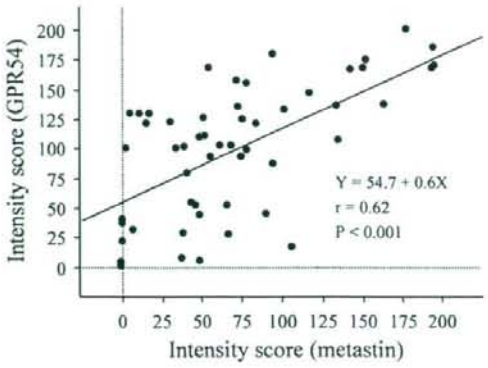

Figure 3
Correlation between metastin and GPR54 expression in pancreatic cancer tissues. Scatter plot showing the correlation between immunoreactivity for metastin and GPR54. A strong correlation was found (r = 0.62, p < 0.001).

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

Table 4: The rate and site of recurrence after resection of pancreatic cancer in relation to GPR54 expression.

	GPR54 expression Positive (n = 30)	GPR54 expression Negative (n = 23)	P value
Recurrence, n (%)	17 (56.7%)	16 (69.6%)	0.34
Site of recurrence			
Liver, n (%)	8 (26.7%)	7 (30.4%)	0.76
Local, n (%)	6 (20.0%)	3 (13.0%)	0.50
Peritoneum, n (%)	5 (16.7%)	4 (17.4%)	0.95
Lymph nodes, n (%)	2 (6.7%)	3 (13.0%)	0.43
Lungs, n (%)	1 (3.3%)	0	0.38
Bone, n (%)	0	1 (4.3%)	0.25
Unknown*, n (%)	0	1 (4.3%)	0.25

* Confirmed by elevated tumor marker during follow-up

both in esophageal squamous cell carcinoma was a significant predictor of lymph node metastasis. Finally, the survival of ovarian cancer patients with low *GPR54* mRNA expression is significantly worse than that of those with high expression[20].

On the other hand, studies in patients with breast cancer[19] and hepatocellular carcinoma (HCC) [15,21] have yielded opposite results, with a positive association between increased KiSS-1 levels and disease progression. Martin et al. [19] found that *KiSS-1* mRNA expression was increased in aggressive breast cancer. Ikeguchi et al. [15] reported that overexpression of *KiSS-1* and *GPR54* was correlated with the progression of HCC. Schmid et al. [21] performed an immunohistochemical study and concluded that high KiSS-1 expression was an independent prognostic factor for shorter survival of patients with HCC.

The mechanism by which the *KiSS-1/GPR54* system regulates tumor progression still remains unclear, although various studies have revealed the downstream signaling pathways activated by *KiSS-1* gene product. This might indicate that a complex signaling network exists with diverse physiological responses [23,28].

Stafford et al. [29] found that binding of *KiSS-1* peptide to the receptor leads to activation of G-protein-activated phospholipase C, which suggested a direct relation of *KiSS-1* to the Gαq-mediated phospholipase C-Ca^{2+} signaling pathway. In addition, activation of GPR54 has been shown to cause an increase of intracellular calcium [9-11],

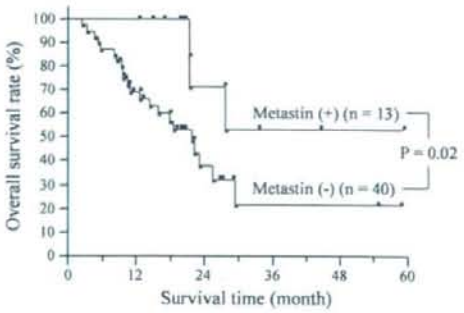

Figure 4
Impact of metastin expression on survival time of pancreatic cancer patients. Overall survival of patients whose tumors were positive (n = 13) or negative (n = 40) for metastin immunostaining. The survival of patients with positive tumors was significantly longer than that of patients with negative tumors (p = 0.02).

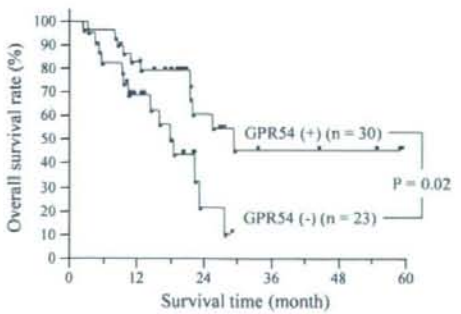

Figure 5
Impact of GPR54 expression on survival time of pancreatic cancer patients. Overall survival of patients whose tumors were positive (n = 30) or negative (n = 23) for GPR54 immunostaining. The survival of patients with tumors positive for GPR54 was significantly longer than that of those with negative tumors (p = 0.02).

Table 5: Univariate and Multivariate analyses of factors associated with survival after resection in patients with pancreatic cancer.

	Univariate analysis		Multivariate analysis	
Characteristics	Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Age (continuous variables)	1.01 (0.97–1.1)	0.50	1.03 (0.97–1.1)	0.29
Gender (male versus female)	1.09 (0.73–1.6)	0.66	1.16 (0.73–1.9)	0.52
Location of tumor (head versus body-tail)	1.08 (0.72–1.7)	0.72	0.71 (0.40–1.3)	0.25
Size of tumor (continuous variables)	1.01 (0.97–1.0)	0.63	1.01 (0.96–1.1)	0.69
Histopathological grading (G1 versus G2-4)	1.05 (0.70–1.7)	0.80	0.92 (0.49–1.8)	0.79
pT (pT1, pT2 versus pT3)	1.62 (0.88–4.0)	0.14	2.07 (0.86–6.7)	0.11
pN (pN0 versus pN1)	1.27 (0.85–2.0)	0.25	1.01 (0.58–1.8)	0.97
Lymphatic invasion (positive versus negative)	1.20 (0.80–1.8)	0.33	0.97 (0.54–1.7)	0.92
Venous invasion (positive versus negative)	1.01 (0.68–1.5)	0.95	0.91 (0.52–1.6)	0.73
Perineural invasion (positive versus negative)	1.57 (1.1–2.4)	0.03	1.47 (0.85–2.7)	0.17
pStage (I, II versus IV)	3.16 (1.6–5.8)	0.002	2.70 (1.1–6.8)	0.03
Residual tumor (R0 versus R1)	1.61 (1.0–2.5)	0.03	1.60 (0.91–2.9)	0.10
Metastin expression (positive versus negative)	1.93 (1.1–4.0)	0.01	2.08 (1.1–4.7)	0.03
GPR54 expression (positive versus negative)	1.62 (1.1–2.5)	0.02	1.22 (0.74–2.0)	0.43

arachidonic acid release [9], activation of mitogen-activated protein kinases (MAPKs), and activation of extracellular signal-regulated kinase (ERK) 1/2[9,14]. We have observed that exogenous metastin reduces migration of pancreatic cancer cells, while it induces the activation of ERK1 and p38[24]. Furthermore, the *KiSS-1* product was shown to repress 92-kDa type 4 collagenase and matrix metalloproteinase (MMP)-9 expression by decreasing the binding of NF-κB to the promoter [30]. Bilban et al. [31] also found downregulation of MMP-2 activity by the *KiSS-1* gene product in human trophoblasts, which implies an association between the tumor suppressor role of *KiSS-1* suggested in this study and our previous report that activation of MMP-2 has a significant role in invasion and metastasis of pancreatic cancer[32].

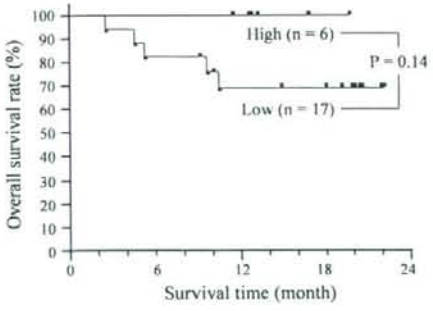

Figure 6
Impact of plasma metastin levels on survival time of pancreatic cancer patients. Overall survival of patients with high (n = 6) and low (n = 17) plasma metastin levels. There was no significant difference between the two groups (p = 0.14), but no patient with a high plasma metastin level died after surgery.

KiSS-1 has also been shown to influence cell adhesion by forming focal adhesions through phosphorylation of focal adhesion kinase and paxillin [11], and an association between loss of KiSS-1 expression and E-cadherin expression was reported in bladder cancer [16].

In our series, there were no significant differences of clinicopathological characteristics between the patients whose tumors showed positive and negative metastin immunostaining, and the result was similar for GPR54. On the other hand, patients whose tumors showed negative immunoreactivity for both metastin and GPR54 had significantly larger tumors than those with lesions positive for either molecule. In addition, recurrence was more frequent in the patients with metastin-negative tumors than in those with metastin-positive tumors. These results suggest that pancreatic cancer loses metastin and GPR54 expression along with its progression. The *KiSS-1* gene is mapped to chromosome 1q32-q41 [33] and *KiSS-1* expression is regulated by genes located on chromosome 6 within the region 6q16.3-q23 [13,28]. These findings are consistent with the fact that loss of 6q, 8p, 9p, 12q, 17p, and 18q is frequently observed in pancreatic cancer[34,35].

Finally, we measured the plasma metastin level in 23 of our patients with pancreatic cancer. We previously found

that the plasma metastin level of patients with pancreatic cancer is significantly higher than that of age- and gender-matched healthy volunteers (unpublished data), so we considered that there was potential to use plasma metastin as a novel tumor marker. In the present series, there was no significant difference of survival between the patients with high and low plasma metastin levels, but no patient with a high plasma metastin level died after surgery. Since the number of patients and the follow-up period are insufficient, more data and further investigation will be needed to clarify the value of measuring plasma metastin.

In this study, the plasma metastin level and metastin immunoreactivity in resected tumor tissues showed a weak correlation. It would be clinically useful if plasma metastin levels had prognostic significance because metastin expression in resected tumor tissues was shown to be a prognostic factor in this study.

Conclusion

In conclusion, expression of metastin and GPR54 was associated with better survival of patients with pancreatic cancer. Metastin expression by cancer tissue was an independent prognostic factor for better survival. Furthermore, the serum metastin level could become a non-invasive prognostic tool for patients with pancreatic cancer.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

KN conceived of the study and performed immunohistochemical studies and measurements of serum metastin. RD conceived of the study, and participated in its design and coordination and helped to draft the manuscript. FK and TI conceived of the study and performed immunohistochemical studies. AK and MK conceived of the study and performed measurements of serum meatstin. TM, YK, KT, SO and NF conceived of the study and performed experiments on pancreatic cancer tissues. SU conceived of the study, and participated in its design.

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Full Paper

Adjuvant 5-fluorouracil and folinic acid *vs* observation for pancreatic cancer: composite data from the ESPAC-1 and -3(v1) trials

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The ESPAC-1, ESPAC-1 plus, and early ESPAC-3(v1) results (458 randomized patients; 364 deaths) were used to estimate the effectiveness of adjuvant 5FU/FA *vs* resection alone for pancreatic cancer using meta-analysis. The pooled hazard ratio of 0.70 (95% CI = 0.55–0.88) $P = 0.003$, and the median survival of 23.2 (95% CI = 20.1–26.5) months with 5FU/FA *vs* 16.8 (95% CI = 14.3–19.2) months with resection alone supports the use of adjuvant 5FU/FA in pancreatic cancer.
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Keywords: pancreatic cancer; gemcitabine; 5-flurouracil; chemoradiation; randomized trial

The results of two recent randomized controlled trials of adjuvant treatment in pancreatic cancer (Oettle *et al*, 2007; Regine *et al*, 2008) have further raised the interest regarding optimum therapy in this disease. The CONK-001 trial showed that postoperative gemcitabine significantly delayed the development of recurrent disease compared with observation alone (Oettle *et al*, 2007) and subsequent analysis showed improved overall median survival (Neuhaus *et al*, 2008). The Radiation Therapy Oncology Group Study (RTOG) 9704 trial showed no difference in the overall survival between two chemoradiotherapy regimens, although in a subgroup analysis showed that the addition of gemcitabine (rather than 5FU) to postoperative adjuvant 5FU-based chemoradiotherapy significantly improved the survival in those patients with cancer in the head of the pancreas (Regine *et al*, 2008).

The European Study Group for Pancreatic Cancer (ESPAC) recruited 550 patients into the ESPAC-1 adjuvant trial (Figure 1) of which 289 patients were in a 2×2 factorial design, powered to investigate the roles of adjuvant chemotherapy (5FU with folinic acid (FA)) and chemoradiotherapy on overall survival (Neoptolemos *et al*, 2001, 2004). The final results confirmed that only adjuvant chemotherapy provided a significant survival benefit (Neoptolemos *et al*, 2004). The trial, however, was not powered for a direct comparison between the 5FU/FA and surgery alone subgroups of the 2×2 design. Of the 550 patients in ESPAC-1, 192 patients were entered into a direct randomised comparison between 5FU/FA and observation alone with clinician's choice of background chemoradiotherapy if indicated. This randomised comparison is referred to as the ESPAC-1 plus trial and was conducted as part of the ESPAC-1 adjuvant trial based on identical eligibility criteria and treatment schedules. Patients were recruited in parallel and in addition to the recruitment target and as such were always intended to be additional evidence not powered for analysis in isolation. The ESPAC-3(v1) trial was initially a three arm study of adjuvant 5FU/FA *vs* gemcitabine *vs* observation. Following the publication of the final results of ESPAC-1 (Neoptolemos *et al*, 2004), the Independent Data Monitoring Committee advised that the observation arm be dropped from ESPAC-3(v2). The Independent Data Monitoring Committee also recommended reporting of the combined results of 5FU/FA *vs* observation from both trials as this was planned as part of the original protocol of ESPAC-3(v1). In the 2×2 component of ESPAC-1 (Figure 1), patients randomised to chemotherapy (either chemotherapy alone or with chemoradiotherapy) were compared with the patients randomised not to receive chemotherapy (either surgery alone or with chemoradiotherapy) as per the 2×2 design, but the unexpected somewhat negative effect of chemoradiotherapy may have affected the result. Hence these data comparing the adjuvant chemotherapy alone *vs* surgery alone subgroups of the 2×2 design are important as a trial including a surgery alone arm is now unlikely to be repeated. The results are thus unique offering for the first time an unbiased randomised comparison of

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adjuvant 5FU/FA *vs* observation following the resection of pancreatic ductal adenocarcinoma. In addition, the use of meta-analysis to combine individual patient data across the three studies increases the overall sample size which, in turn, increases the statistical power of the analysis.

METHODS

The inclusion criteria in ESPAC-1, ESPAC-1 plus, and ESPAC-3(v1) were identical and postoperative restaging and CA 19.9 values were not used to determine patient inclusion in these studies (Neoptolemos *et al*, 2001, 2004; www.cancernorth.nhs.uk/portal_repository/files/trial_sum_espac.pdf). Similarly, the chemotherapy regimen used was identical in all three studies comprising an intravenous bolus of leucovorin (folinic acid; 20 mg m^{-2}), followed by an intravenous bolus of 5FU (425 mg m^{-2}) on each of 5 consecutive days every 28 days for six cycles. There were 144 patients from the two groups of the ESPAC-1 2 × 2 design (69 observation, 75 5FU/FA) with a median follow-up of the 24 alive patients of 78 (interquartile range = 45–92) months (Table 1). The ESPAC-1 plus component recruited 192 patients (95 observation, 19 (20%) of whom received background chemoradiotherapy; 97 5FU/FA, 25 (26%) of whom received background chemoradiotherapy) with a median follow-up of the 40 alive patients of 64 (interquartile range = 20–89) months. There were 122 patients in ESPAC-3(v1) at closure of the observation arm in this trial (61 observation, 61 5FU/FA) with a median follow-up of the 30 alive patients of 54 (interquartile range = 34–60) months. These data provide a direct randomised comparison of 5FU/FA *vs* observation alone based on the intention-to-treat principle. For the outcome of overall survival, a random effects model was used to combine the trial level hazard ratios (HRs), estimated from the individual patient data, using an inverse variance meta-analysis. Survival estimates are presented as simple, non-stratified Kaplan–Meier curves across all trials. The overall estimate of the treatment effect is adjusted by any influence of trial.

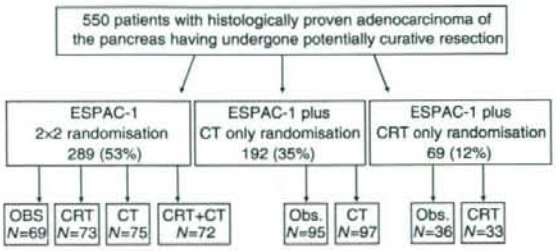

Figure 1 ESPAC-1 trial design.

RESULTS

The eligibility criteria across trials were similar, and as such the patient and tumour characteristics (Table 1) were comparable with treatment schedules also identical across trials. At the time of analysis, there were 120 (83.3%) deaths in ESPAC-1, 152 (79.2%) deaths in ESPAC-1 plus, and 92 (75.4%) deaths in ESPAC-3(v1) (Table 2). The heterogeneity between trials was non-significant, and pooling the data is considered justifiable (Figures 2 and 3). The overall survival (Figure 4) was superior in patients randomized to 5FU/FA compared to those randomized to observation (pooled HR = 0.70 (95% CI = 0.55–0.88); $P = 0.003$ (Table 2)) with evidence of low statistical heterogeneity ($P = 0.27$, $I^2 = 25\%$, Figure 3). The pooled effect of chemotherapy is estimated to reduce the risk of death by 30% compared to surgery alone. Combined overall median survival (obtained from simple Kaplan–Meier curves non-stratified by trial) was 23.2 (95% CI = 20.1–26.5)

Table 1 Patient characteristics and observation of patients randomised to 5FU/FA

	ESPAC-1 (*N* = 144)		ESPAC-1 plus (*N* = 192)		ESPAC-3 (*N* = 122)		Total
	Obs. (*N* = 69)	5FU/FA (*N* = 75)	Obs. (*N* = 95)	5FU/FA (*N* = 97)	Obs. (*N* = 61)	5FU/FA (*N* = 61)	*N* = 458
Sex:							
Male	47 (68%)	44 (59%)	54 (57%)	60 (62%)	40 (66%)	34 (56%)	279 (61%)
Female	22 (32%)	31 (41%)	41 (43%)	37 (38%)	21 (34%)	27 (44%)	179 (39%)
Age:							
Median (years)	60	61	60	57	62	61	60
IQR	55–65	55–67	54–69	51–63	53–69	55–67	54–67
Range	36–84	41–83	32–84	28–78	33–77	42–80	28–84
Max. tumour size:							
Median (cm)	3.0	3.0	3.0	3.0	2.9	2.8	3.0
IQR	2.0–3.5	2.5–4.0	2.3–3.5	2.1–4.0	2.0–3.5	2.0–3.3	2.2–3.5
Range	0.6–5.0	0.6–8.0	0.5–9.0	0.6–10.0	1.0–6.0	0.3–6.0	0.3–10.0
Grade:							
Well	12 (18%)	21 (31%)	19 (20%)	18 (20%)	5 (8%)	11 (18%)	86 (20%)
Moderate	40 (62%)	28 (42%)	52 (56%)	57 (62%)	43 (70%)	30 (50%)	250 (57%)
Poor	13 (20%)	18 (27%)	22 (24%)	17 (18%)	12 (20%)	18 (30%)	100 (23%)
Undifferentiated	0	0	0	0	1 (2%)	1 (2%)	2 (0%)
Lymph nodes:							
Neg.	25 (37%)	35 (49%)	51 (56%)	48 (52%)	21 (34%)	18 (30%)	198 (45%)
Pos.	42 (63%)	36 (51%)	40 (44%)	45 (48%)	40 (66%)	42 (70%)	245 (55%)
Resection margins:							
Neg.	60 (87%)	61 (81%)	73 (77%)	74 (76%)	38 (62%)	37 (61%)	343 (75%)
Pos.	9 (13%)	14 (19%)	22 (23%)	23 (24%)	23 (38%)	24 (39%)	115 (25%)

Table 2 Survival estimates

Comparison	Number of patients	Number of deaths	Median survival in months (95% CI)	Survival rates at 1, 2, and 5 years	Hazard ratio (95% CI)
ESPAC-1	144	120	18.6 (15.7, 23.6)	67%, 42%, 18%	1.0
ESPAC-1 plus	192	152	17.4 (15.8, 21.7)	66%, 38%, 19%	1.03 (0.81, 1.32)[a]
ESPAC-3	122	92	24.3 (19.8, 30.9)	80%, 51%, 20%	0.86 (0.66, 1.11)[a]
Overall	458	364	19.6 (17.3, 22.0)	70%, 43%, 19%	—
ESPAC-1					
Obs	69	63	16.9 (12.3, 24.8)	64%, 39%, 10%	1.0
5FU/FA	75	57	21.7 (14.8, 27.3)	70%, 44%, 27%	0.70 (0.49, 1.01)
ESPAC-1 plus					
Obs.	95	80	12.8 (10.2, 16.9)	52%, 28%, 14%	1.0
5FU/FA	97	72	24.0 (18.8, 29.4)	81%, 49%, 24%	0.58 (0.42, 0.80)
ESPAC-3					
Obs.	61	47	20.3 (18.1, 31.7)	79%, 48%, 20%	1.0
5FU/FA	61	45	25.9 (18.3, 36.3)	82%, 54%, 20%	0.89 (0.59, 1.33)
Overall					
Obs.	225	190	16.8 (14.3, 19.2)	63%, 37%, 14%	—
5FU/FA	233	174	23.2 (20.1, 26.5)	77%, 49%, 24%	**0.70 (0.55, 0.88)**[b]

[a]$P_{LR} = 0.33$. [b]Adjusted by trial. Bold value signifies $P = 0.003$.

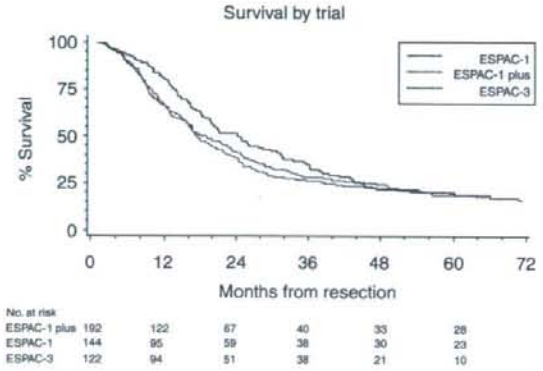

Figure 2 Kaplan–Meier survival curves stratified by trial.

months for 5FU/FA compared to 16.8 (95% CI = 14.3–19.2) months for observation with 2- and 5-year survival estimates of 49%, 24% for 5FU/FA and 37%, 14% for observation (Figures 4 and 5, Table 2). A sensitivity analysis excluding the ESPAC-1 plus study estimated that chemotherapy reduced the risk of death by 23% compared to surgery alone (HR = 0.77, 95%CI = 0.59, 1.01).

DISCUSSION

This individual patient data meta-analysis of ESPAC-1, ESPAC-1 plus and ESPAC-3 trials showed significantly better overall survival for patients randomized to 5FU/FA with an HR of 0.70 (95% CI = 0.55, 0.88; $P = 0.003$) indicating a significant reduction in the risk of death of 30% with 5FU/FA compared with surgery alone.

The CONKO-001 trial (Oettle *et al*, 2007) found a significantly improved median disease-free survival in favour of gemcitabine (13.4 (range = 11.4–15.3) months) compared to observation (6.9 (range = 6.1–7.8) months; $P < 0.001$). The overall median survival was 22.1 (range = 18.4–25.8) months for the gemcitabine group, and 20.2 (range = 17–23.4) months for the surgery alone group (HR = 0.79 (95% CI = 0.62–1.01); $P = 0.06$). The primary end point was disease-free survival, whereas a confounding factor for overall survival was the fact that a large proportion of the control group received gemcitabine on relapse. The CONKO-001 investigators concluded that chemotherapy with gemcitabine offered the best benefit/risk ratio of all currently available adjuvant treatment options (Oettle *et al*, 2007). Comparison with the current study using an adjusted indirect comparison, which maintains the within trial randomisation (Bucher *et al*, 1997) shows that the adjuvant 5FU/FA has at least similar survival results to those of gemcitabine (adjusted indirect HR of 0.89 (95% CI = 0.63–1.25) for 5FU compared with gemcitabine), although equivalence cannot be claimed due to the wide confidence interval and should be interpreted cautiously as not as reliable as a direct comparison. Furthermore, the toxicity for gemcitabine in the CONKO-001 trial appears less than that for 5FU/FA (Neoptolemos *et al*, 2001, 2004), but a robust assessment of the benefit/risk ratio can only be properly addressed by a concurrently randomised comparison as will be carried out in ESPAC-3.

The RTOG-9704 trial compared pre and postchemoradiation gemcitabine ($1000\,mg\,m^{-2}\,day^{-1}$) to pre and postchemoradiation 5FU ($250\,mg\,m^{-2}\,day^{-1}$ given as a continuous infusion) in patients who had undergone pancreatic resection (Regine *et al*, 2008). Both arms of the study received 5FU-based chemoradiotherapy (50.4 Gy), with the chemotherapy given for 3 weeks pre- and 12 weeks postchemoradiotherapy (Regine *et al*, 2008). Analysis was restricted to 442 'eligible' patients out of the total of 538 patients originally recruited. There was no difference in the overall survival between the two arms, but a prospectively powered subgroup analysis of the 380 patients with pancreas head cancer revealed a reduction in the risk of death for patients in the gemcitabine-based chemoradiation arm (HR = 0.79; 95% CI = 0.63–0.99; $P = 0.047$). The conclusions of the ESPAC-1 trial and subsequent meta-analyses with other adjuvant trials suggest that there is no good clinical evidence for the use of chemoradiation in pancreatic cancer in the adjuvant setting (Neoptolemos *et al*, 2001, 2004; Stocken *et al*, 2005) or in patients with locally advanced disease (Yip *et al*, 2006; Sultana *et al*, 2007a, b), and more recent results are conflicting (Chauffert *et al*, 2008; Loehrer *et al*, 2008). The apparent failure of chemoradiation in pancreatic

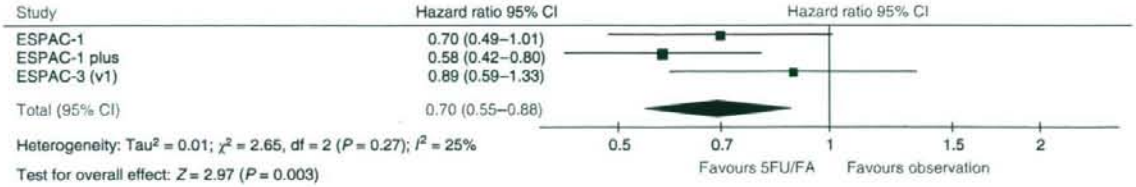

Figure 3 Meta-analysis of ESPAC-1, ESPAC-1 plus ESPAC-3 (v1) trials for overall survival.

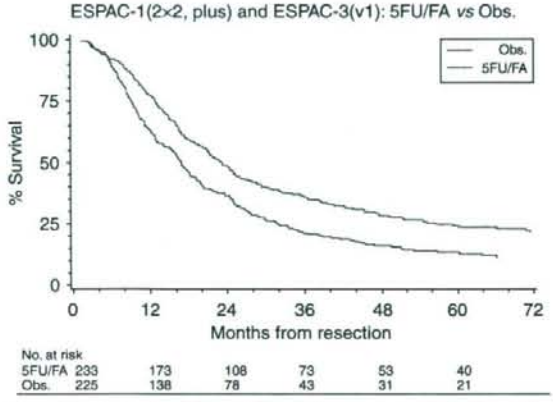

Figure 4 Kaplan–Meier overall survival curves non-stratified by trial.

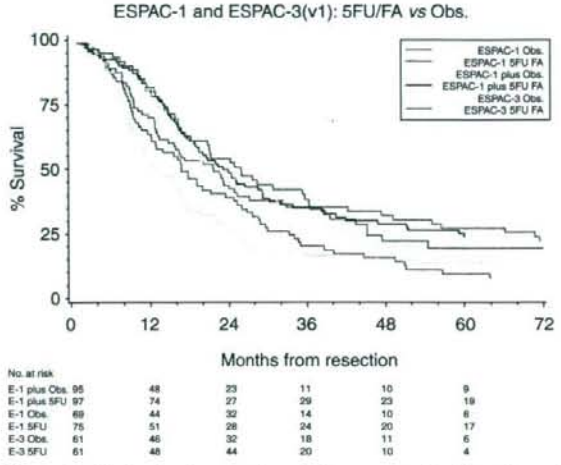

Figure 5 Kaplan–Meier overall survival curves stratified by trial and treatment group.

cancer may be ascribed to interference of systemic chemotherapy scheduling and/or significant biological effects, such as the prometastasizing effects of ionising radiation (Biswas *et al*, 2007).

In conclusion, the current evidence supports the continued use of adjuvant 5FU/FA for treating pancreatic cancer. The results of the ESPAC-3(v2) trial will determine whether gemcitabine is superior or not to this treatment.

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