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

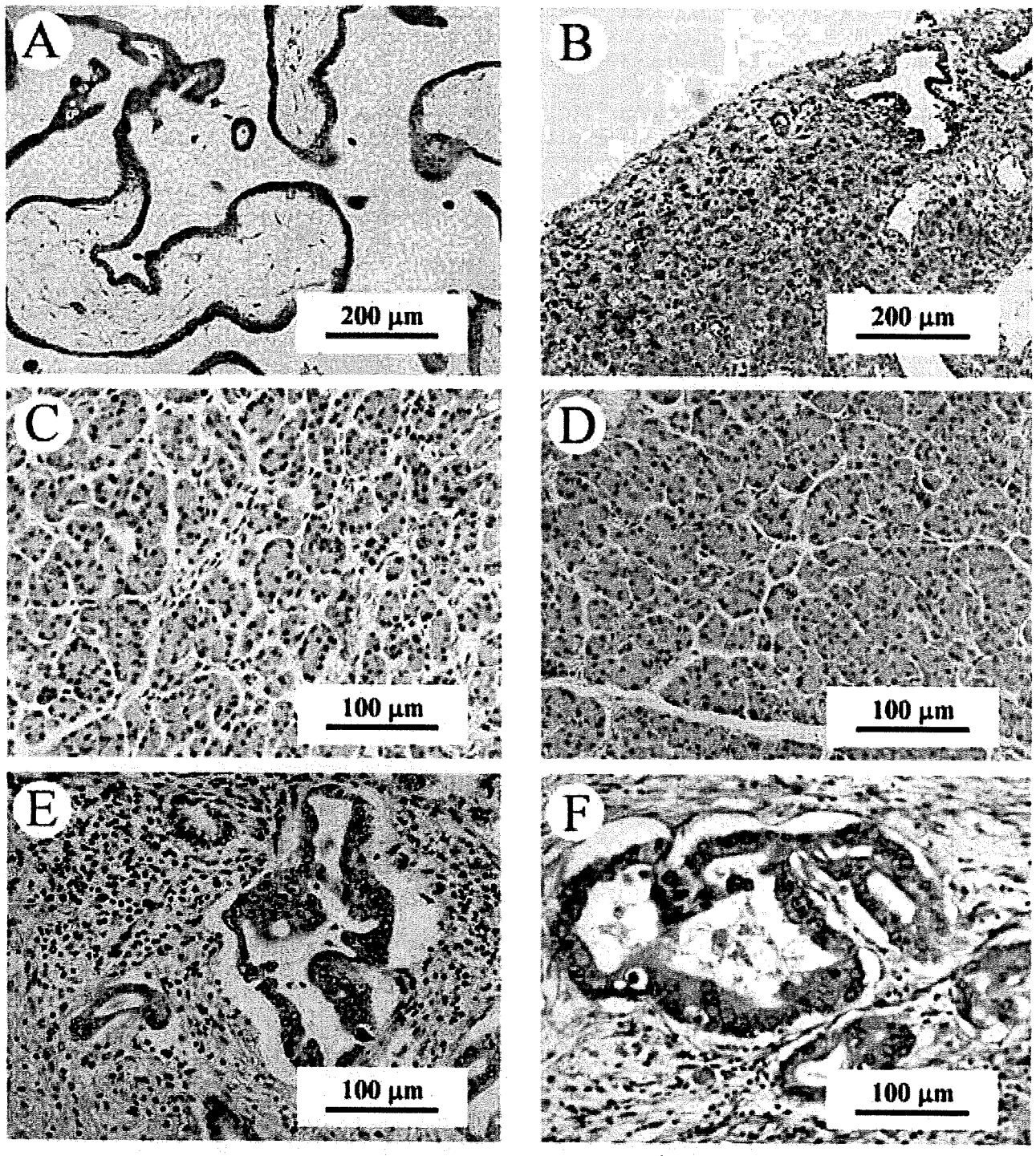

Figure 1
Immunohistochemical staining of non-cancerous pancreatic tissues and pancreatic cancer tissues. (A, B); Immunohistochemical staining of human placental tissues as a positive control. Tissues were stained with anti-metastin (A) and anti-GPR54 antibody (B). (Original magnification, × 200). (C, 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.

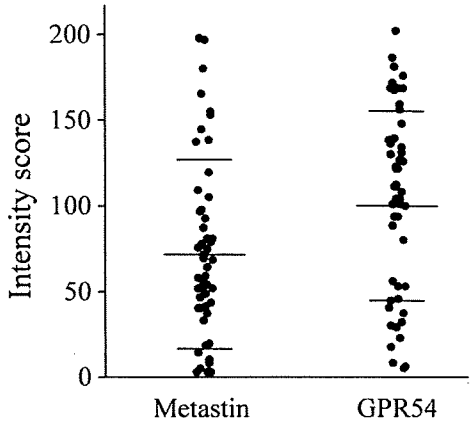

Figure 2
Expression of metastin and GPR54 in pancreatic cancer tissues. Immunoreactivity for metastin and GPR54 in resected pancreatic cancer tissues (n = 53) shown as the intensity score of each patient. The mean metastin intensity score was 72.1 ± 54.9 and that for GPR54 was 99.9 ± 55.1. The horizontal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

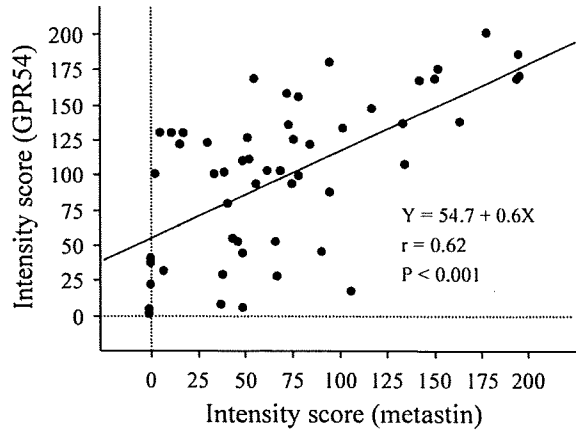

Figure 3
Correlation between metastin and GPR54 expression in pancreatic cancer tissues. Scatter plot showing the correlation between immunoreactivity for metastin and GPR54. A strong correlation was found ($r = 0.62$, $p < 0.001$).

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],

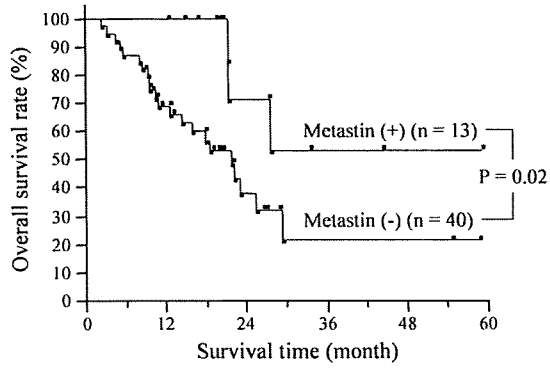

Figure 4
Impact of metastin expression on survival time of pancreatic cancer patients. Overall survival of patients whose tumors were positive (n = 13) or negative (n = 40) for metastin immunostaining. The survival of patients with positive tumors was significantly longer than that of patients with negative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

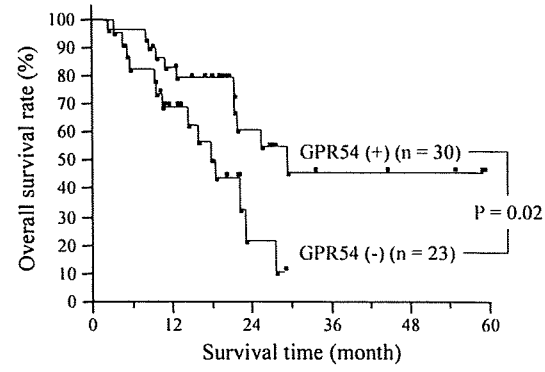

Figure 5
Impact of GPR54 expression on survival time of pancreatic cancer patients. Overall survival of patients whose tumors were positive (n = 30) or negative (n = 23) for GPR54 immunostaining. The survival of patients with tumors positive for GPR54 was significantly longer than that of those with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

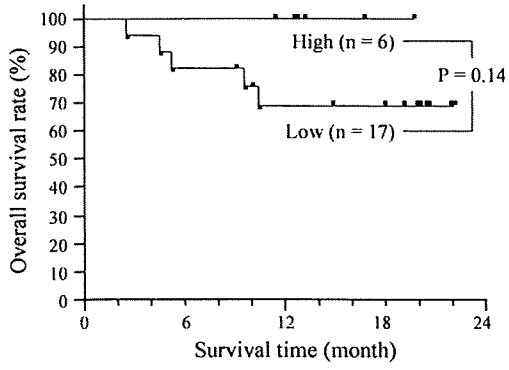

Figure 6
Impact of plasma metastin levels on survival time of pancreatic cancer patients. Overall survival of patients with high (n = 6) and low (n = 17) plasma metastin levels. There was no significant difference between the two groups (p = 0.14), but no patient with a high plasma metastin level died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

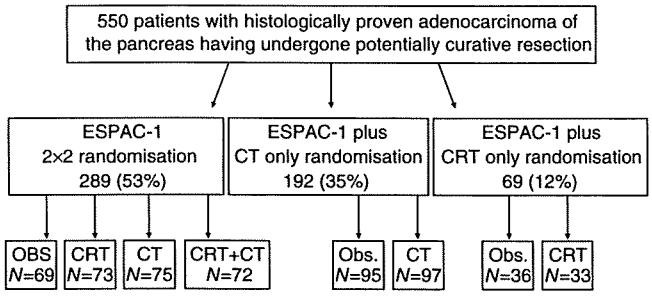

Figure 1 ESPAC-1 trial design.

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 $P = 0.003$.

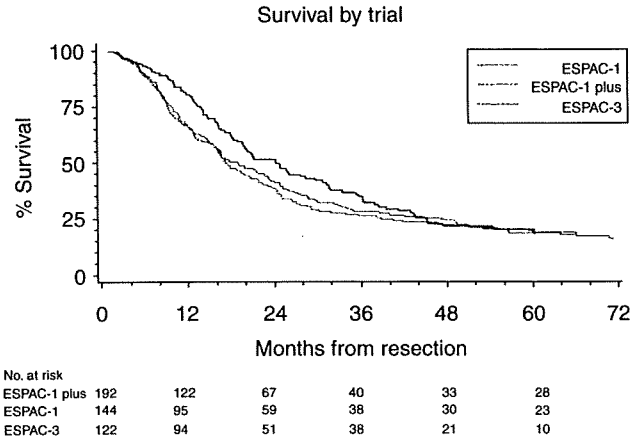

Figure 2 Kaplan–Meier survival curves stratified by trial.

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

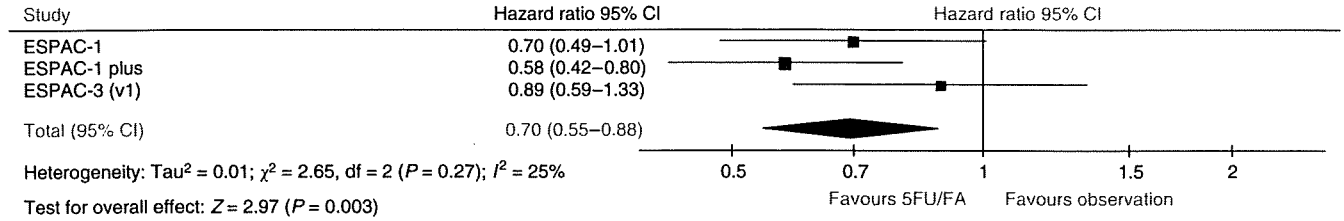

Figure 3 Meta-analysis of ESPAC-1, ESPAC-1 plus ESPAC-3 (v1) trials for overall survival.

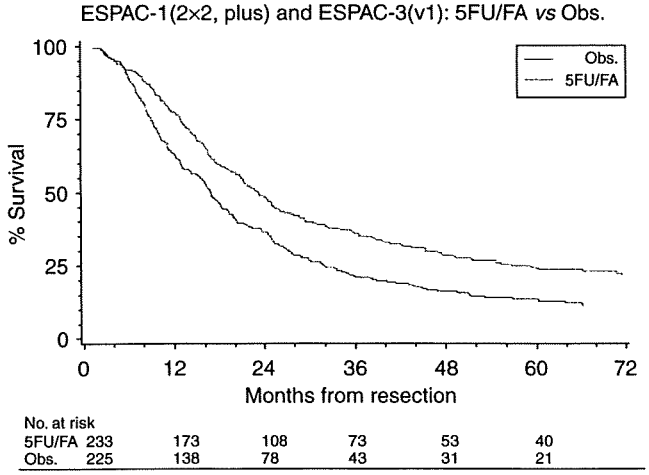

Figure 4 Kaplan–Meier overall survival curves non-stratified by trial.

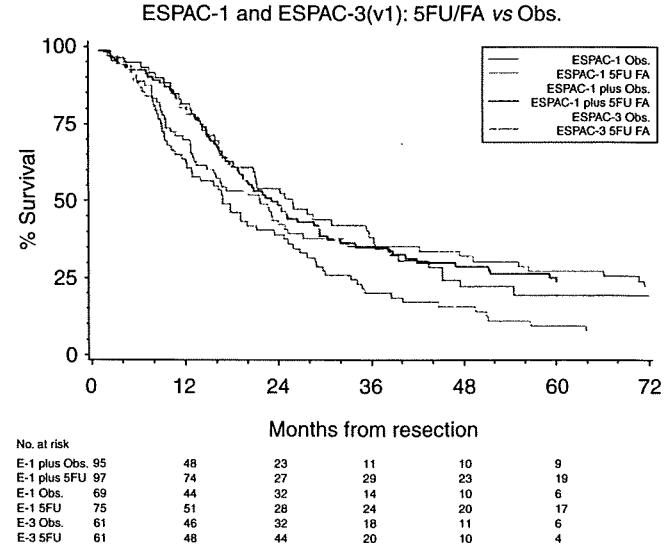

Figure 5 Kaplan–Meier overall survival curves stratified by trial and treatment group.

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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SURGERY TODAY

Surgery Versus Radiochemotherapy for Resectable Locally Invasive Pancreatic Cancer: Final Results of a Randomized Multi-Institutional Trial

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Abstract

Purpose. Although the outcome of surgery for locally advanced pancreatic cancer remains poor, it is improving, with 5-year survival up to about 10% in Japan. The preliminary results of our multi-institutional randomized controlled trial revealed better survival after surgery than after radiochemotherapy. We report the final results of this study after 5 years of follow-up.

Methods. Patients with preoperative findings of pancreatic cancer invading the pancreatic capsule without involvement of the superior mesenteric or common hepatic arteries, or distant metastasis, were included in this randomized controlled trial, with their consent. If the laparotomy findings were consistent with these criteria, the patient was randomized to a surgery group or a radiochemotherapy group (5-fluorouracil 200 mg/m^2/day and 5040 Gy radiotherapy). We compared the mean survival time, 3- and 5-year survival rates, and hazard ratio.

Results. The surgery and radiochemotherapy groups comprised 20 and 22 patients, respectively. Patients were followed up for 5 years or longer, or until an event occurred to preclude this. The surgery group had significantly better survival than the radiochemotherapy group ($P < 0.03$). Surgery increased the survival time and 3-year survival rate by an average of 11.8 months and 20%, respectively, and it halved the instantaneous mortality (hazard) rate.

Conclusion. Locally invasive pancreatic cancer without distant metastases or major arterial invasion is treated most effectively by surgical resection.

Key words Pancreatic cancer · Local invasion · Radiochemotherapy · Randomized multi-institutional trial · Laparotomy · Long-term survival

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Introduction

Pancreatic cancer is difficult to resect curatively. The results of surgical treatments, including super-radical resections, are still poor.[1–3] Pancreatic cancer is now the fifth leading cause of cancer death in Japan, killing more than 20,000 persons every year in this country.[4,5] The overall 5-year survival rate after radical curative surgery ranges from 6.8% to 25%.[6–12] Nevertheless, many surgeons believe that a strategy including curative intent resection is the only way to achieve long-term survival for pancreatic cancer patients, considering that improvements in operative and perioperative management over the past 20 years have led to a decrease in operative mortality and a shorter hospital stay.[6,7,13]

Pancreatic cancer is also considered to be one of the most chemo-resistant human malignancies.[14–16] The results of a few randomized controlled trials suggest that concomitant external beam radiotherapy and chemotherapy (radiochemotherapy) is more effective than chemotherapy alone or radiation therapy alone for patients with advanced non-resectable pancreatic cancer without distant metastasis.[17–19] However, there is no consensus on the treatment strategy for locally advanced pancreatic cancer without distant metastasis because, to our knowledge, no randomized controlled trial has been conducted for patients with this stage of pancreatic cancer. Thus, we conducted our own randomized controlled trial to establish the best treatment strategy for locally advanced pancreatic cancer extending beyond the pancreatic capsule without invasion of the superior mesenteric artery or the common hepatic artery. Although our preliminary data indicated a survival benefit for surgery over radiochemotherapy, the results were inconclusive because of the short follow-up.[20] In this report, we summarize the final results of this trial after it reached the primary end point and all patients had been followed up for 5 years or longer, or until they died.

Patients and Methods

Eligibility

The following patient enrolment criteria were established based on the definition of Stage IVa cancer according to the Japanese classification system version 4 (JCS)[21]:

1. Age between 20 and 75 years, with a performance status (PS) of 0–2
2. Tumor invasion of either the serosal (anterior) or retroperitoneal (posterior) surface of the pancreas, or extension into the intrapancreatic portal vein without complete obstruction; defined as S2, RP2, or PV2 according to the JCS
3. No involvement of adjacent organs, apart from the transverse mesocolon, duodenum, and common bile duct
4. No invasion of the superior mesenteric artery, the common hepatic artery, or the peripancreatic nerve plexuses (A0 and PL0)
5. No para-aortic lymph node metastasis (N0 or N1)
6. Greatest diameter of the tumor within the range of 2–6 cm (TS2 or TS3)
7. No liver metastasis or peritoneal seeding (H0 and P0)

The other exclusion criteria were a history of radiation therapy or chemotherapy, idiosyncrasy to drugs including contrast media, the coexistence of serious cardiovascular, pulmonary, renal or hepatic diseases, a concurrent active neoplasm, and any other condition that we considered could preclude the trial.

The schema for the current study protocol is shown in Fig. 1. When the above eligibility criteria were met, based on the findings of preoperative examinations including abdominal computed tomography (CT), angiography and ultrasonography, chest X-rays, and routine laboratory tests, and after giving written informed consent to be part of the trial, the patient was registered as a potential candidate at the central office not later than 1 day before the scheduled laparotomy. The final eligibility decision was based on the operative findings by laparotomy, and the patient was randomized by a telephone call to the central office.

Randomization

The 19 participating institutions, located all over Japan, were grouped into seven blocks according to district. Randomization was done separately in each block. Patients deemed to be eligible according to the operative findings were randomly allocated to one of the two treatment groups. When equal numbers of patients in the stratum (tumor location) were assigned in both treatment groups, new patients were randomized to either treatment. If the number of patients in one treatment group differed by more than two, new patients were assigned to the smaller treatment group according to stratified block randomization.

Treatments

Patients assigned to the surgery group underwent pancreatoduodenectomy (PD) or distal pancreatectomy for resection of the main pancreatic cancer, with dissection of the Group 1 regional lymph nodes or more according to the JCS.[21] At least a half-circle of the plexus of the

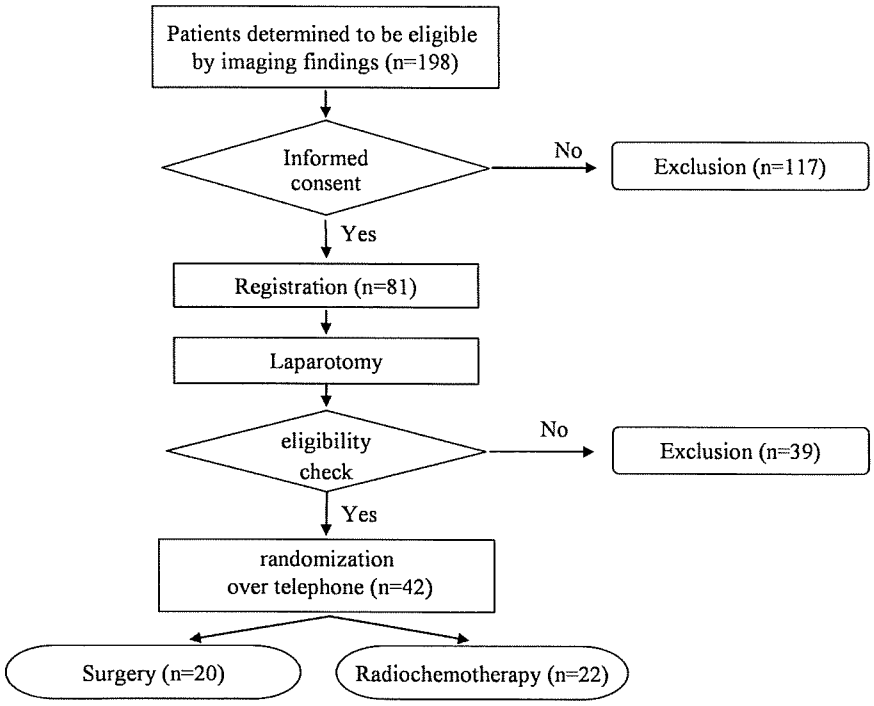

Fig. 1. Schema for the study protocol

root of the superior mesenteric artery was resected. Patients were not given adjuvant therapy postoperatively until recurrence became evident, at which point the doctor in charge was permitted to select another treatment.

In patients assigned to the radiochemotherapy group, the abdomen was closed after a biopsy specimen was taken for confirmation of the diagnosis, although the surgeon in charge was permitted to perform anastomotic surgery such as gastrojejunostomy or biliodigestive anastomosis. Within 1 week, the patient received X-ray irradiation. Radiation therapy was delivered as a single course, to a total radiation dose of 5040 cGy, in 28 fractions at 180 cGy over 5.5 weeks, using 10–14 MV photons. The radiation field included the primary tumor and a margin of 1–3 cm covering the regional lymph nodes, and was defined by treatment-planning computed tomography obtained 1–2 days prior to treatment. Lateral treatments were administered together with anteroposterior:posteroanterior (AP:PA) fields so that radiation to the spinal cord could be limited to 4000 cGy. A continuous intravenous infusion of 5-fluorouracil (5-FU) at 200 mg/m^2/day was maintained throughout the radiation therapy. After finishing the regimen, these patients were given an intravenous infusion of 5-fluorouracil (5-FU), 500 mg/m^2, weekly, usually starting within 1 week and at least within 4 weeks of completion of the radiochemotherapy.

Statistical Analysis

The sample size was calculated as follows. Assuming that the 1-year survival rate for Stage IVa cancer treated by surgical resection is 60%, whereas the 1-year survival for locally advanced cancer treated by radiochemotherapy is 40% (median survival of 9 months), we needed 73 patients per group in order to detect the difference at a one-sided 5% significance level with 20% power. Thus, the target sample size was set at 150 patients. Both treatments were done as routine procedures with unpredictable complications or death considered unlikely. We scheduled interim analysis for when half the target sample size was reached.

The distributions of the patients' baseline characteristics in the two treatment groups were compared using the chi-square test for binary variables, the Mann–Whitney *U*-test for ordinal variables, and the unpaired *t*-test for continuous variables. The conventional survival statistics, including the hazard ratio (log rank test) and 3- and 5-year survival rates, were calculated to compare the outcomes of the two treatment groups. In addition to the median survival time, the mean survival time was also estimated since it has recently been recognized as a better indicator of survival benefits.[22–24] This was calculated as the area under the survival curve[25] and its standard error was estimated using the Irwin method[26] with Kaplan–Meier adjustment[27] for the total

Table 1. Patient background

	Surgery group ($n = 20$)	Radiochemotherapy group ($n = 22$)	P value
Age (mean [range])	64.7 [51–75]	62.6 [49–72]	1.66
Sex			
Male	12	15	0.39
Female	8	7	

number of deaths. To assess the prognostic significance of individual variables and to identify independent predictors of survival, we used Cox regression analysis and a stepwise selection procedure.

Postoperative change in quality of life scores,[28] based on performance status, general well-being, diarrhea, and pain; and laboratory data, comprising hemoglobin, total protein, albumin, total cholesterol, carcinoembryonic antigen (CEA), and carbohydrate antigen (CA 19-9) levels, were compared using a repeated measure analysis of variance between the treatment groups. The Statistical Package for Social Science (SPSS, version 11; SPSS, Chicago, IL, USA) was used for these analyses.

Results

Patient Characteristics

During the study period, resectable locally advanced pancreatic cancer meeting the criteria of the study protocol was diagnosed in 198 patients in the participating institutes. These patients were fully informed about the study, and asked if they would register for the clinical trial. Written informed consent was obtained from 81 (41%) of these patients: the remaining 117 were not registered in the clinical trial because they asked specifically to be treated with surgery ($n = 91$) or radiochemotherapy ($n = 26$).

Because the required number of patients was not enrolled in the first 2 years, accrual was extended by another 2 years. We performed an interim analysis after 4 years, which showed a clear survival benefit for the surgery group, so enrollment and registration for the trial was closed. Of the original 81 potentially eligible patients who were registered and underwent laparotomy, 39 were excluded because laparotomy revealed distant metastasis or peritoneal metastasis in 10 patients, distant lymph node metastases in 9 patients, liver metastasis in 6 patients, and invasion of adjacent structures including the anterior or retroperitoneal organs, the superior mesenteric artery, or nerve plexuses in 16 patients. Three patients were excluded from the study because the operative findings confirmed stage III disease, and one patient was excluded because the lesion was smaller than 2 cm in diameter.

We compared the preoperative evaluation based on imaging findings with the operative findings in all registered patients. Computed tomography evaluation had limited diagnostic accuracy of 65% for anterior capsular invasion, 84% for retroperitoneal invasion, and 86% for portal venous system invasion.

The remaining 42 patients (Table 1) were randomized and treated as indicated, with 20 patients assigned to the surgery group (12 men and 8 women; average age, 64.7 years), and 22 patients assigned to the radiochemotherapy group (15 men and 7 women; average age, 62.6 years). There were no significant differences in their backgrounds. The patients in the surgery group underwent surgical resection, consisting of PD in 15 patients (in the form of PD in 8 and pylorus-preserving PD (PPPD) in 7), and distal pancreatectomy (DP) in 4 patients. One patient in the surgery group was found to have extensive invasion of the superior mesenteric artery, and resection was discontinued based on the surgeon's judgment. This patient was subsequently treated with radiochemotherapy, but included in the surgery group on a treatment-intended basis. The mode of operation and the level of lymph node and nerve plexus dissection are shown in Table 2.

All 22 patients in the other group received radiochemotherapy after the laparotomy; however, both the radiation and 5-FU were discontinued in three patients; because of severe colitis in one, disease progression in one, and refusal of treatment in one. The doses of radiation and 5-FU given to these patients are summarized in Table 3.

Survival

The baseline variables, apart from lymph node metastasis, were comparable in the two groups (Table 4). Because lymph node dissection was not performed in the radiochemotherapy group, the evaluation of the lymph node metastasis was different in each group. The survival curves of the two treatment groups are shown in Fig. 2. The mean survival time was significantly longer after surgery than after radiochemotherapy, with a mean difference of 11.8 months and a hazard ratio of 0.46 (95% confidence interval: 0.22–0.92; Table 5). The 3-year survival rate after radiochemotherapy was 0%, whereas after surgery, it was 20% ($P = 0.025$). Similarly, the 5-year survival rate after radiochemotherapy was 0%, whereas after surgery, it was 10%, although this difference was not significant. The Cox univariate

analyses revealed that among the variables, treatment was the only significant predictor of survival and the only independent predictor.

Effects of Treatment on Quality of Life Scores and Other Variables

The mean hospital stay for the surgery group was significantly shorter than that for the radiochemotherapy group, at 66 days vs 102 days ($P = 0.03$; Table 6). The Japanese insurance system generally allows patients to remain in hospital until they are able to live independently in their homes without professional support. The total costs for the primary hospital stay were \$17500 ± \$5120 for surgery with postoperative care, and \$28200 ± \$6130 for radiochemotherapy (mean ± SD).

Both treatments resulted in significant decreases in body weight, hemoglobin, albumin, and total cholesterol levels 3 months after laparotomy, and the patients' level of satisfaction was significantly increased in both groups. The degrees of these changes did not differ significantly between the two groups. There was a significant difference only in the average number of bowel movements per day, which increased after surgery but remained unchanged after radiochemotherapy. There was no significant difference in the performance status or pain score, or in the changes of the serum concentrations of CEA and CA 19-9 between the groups.

Table 2. Treatment results in the surgery group

	No. of patients
Mode of operation	
PD	8
PPPD	7
DP	4
Lymph node dissection	
D2	9
D1α	10
Resection of PL ph1	
Complete	16
Incomplete	0
None	3
Resection of PL ph2	
Complete	16
Incomplete	0
None	3
Resection of PL sma	
Complete	4
Incomplete	13
None	2
Resection of PL ce	
Complete	1
Incomplete	15
None	3
Reconstruction	
Whipple method	4
Child or modified	10
Imanaga method	5

The following abbreviations were taken from the Japan Pancreas Society classification of pancreatic carcinoma (version 4)[21]: PD, pancreatoduodenectomy; PPPD, pylorus-preserving pancreatoduodenectomy; D2, lymph node which belong to group 2; D1α, lymph node which belong to group 1 or more; PL ph1, pancreatic head plexus I; PL ph2, pancreatic head plexus II; PL sma, superior mesenteric arterial plexus; PL ce, celiac plexus

Discussion

It is well known that the survival rate of patients with pancreatic cancer is much lower than that of patients with other gastrointestinal cancers.[13,29] Thus, at what stage of pancreatic cancer would patients benefit from surgical resection? This has been a subject of much discussion and yet, a world-wide consensus has not been reached with no randomized controlled trial performed on this subject. The results of the current study and those of our preliminary analysis[20] provide solid evidence that surgery is much more effective than radiochemotherapy alone for resectable locally-advanced

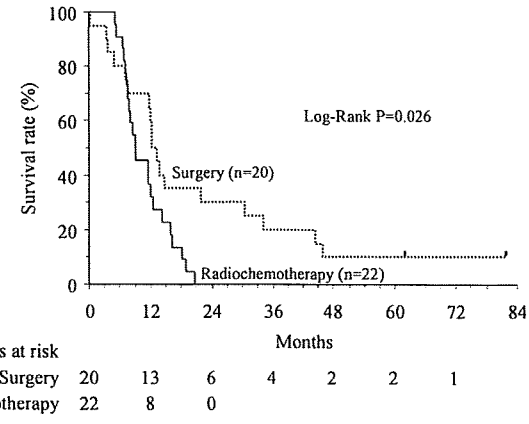

Fig. 2. Survival curves for patients treated with curative-intent surgery and those treated with radiochemotherapy. All patients were followed up for 5 years or longer

Table 3. Treatment results in the radiochemotherapy group

Total dose of radiation (cGy)	4518 ± 1420
Total dose of 5-fluorouracil during radiation therapy (mg)	9805 ± 4429
Total dose of 5-fluorouracil following radiation therapy (mg)	10114 ± 4766

Values are expressed as means ± standard deviation