Table 4. Laparotomy findings of primary tumor extension

	Surgery group ($n = 20$)	Radiochemotherapy group ($n = 22$)	P value
Localization			
Head	16	18	0.64
Body/tail	4	4	
Size (cm)			
2–4	11	14	0.38
4–6	9	8	
Invasion of the anterior pancreatic capsule			
S(−)	10	10	0.56
S(+)	10	12	
Invasion of the retroperitoneal tissue			
RP(−)	8	11	0.34
RP(+)	12	11	
Invasion of the portal venous system			
PV(−)	4	7	0.24
PV(+)	16	15	
Invasion of the arterial system			
A(−)	19	21	0.66
A(+)	1	1	
Invasion of the distal bile duct			
CH(−)	7	9	0.50
CH(+)	13	13	
Invasion of the duodenal wall			
DU(−)	13	12	0.11
DU(+)	7	10	
Invasion of the extrapancreatic nerve plexuses			
PL(−)	18	21	0.23
PL(+)	2	1	
Lymph node metastasis			
N(−)	6	17	<0.001
N(+)	14	5	

Values are expressed as the number of patients

Table 5. Comparison of survival in the two treatment groups

	Radiochemotherapy group	Surgery group	Difference	P value
3-year survival (%)	0	20	20	0.025
(95% CI)	(0–0)	(3–38)	(3–38)	
5-year survival (%)	0	10	10	0.136
(95%CI)	(0–0)	(−3–23)	(−3–23)	
Median survival (months)	8.9	12.1	3.2	0.161
(95% CI)	(5.1–12.7)	(9.7–14.4)	(−1.3–7.7)	
Mean survival (months)	10.8	22.6*	11.8*	0.025*
(95% CI)	(8.8–12.7)	(12.5–32.7)*	(1.5–22.1)*	
Hazard ratio			0.46	0.026
(95% CI)			(0.22–0.92)	

*Value at the time of analysis with two survivors in the surgery group

Table 6. Length of hospitalization

	Surgery group ($n = 20$)	Radiochemotherapy group ($n = 22$)	P value
Initial hospitalization (days)	66 ± 29	101 ± 57	0.03*
Total hospitalization (days)	101 ± 60	124 ± 59	0.28

Values are expressed as means ± standard deviation
*This value was significant

pancreatic cancer extending beyond the pancreatic capsule, but not invading the superior mesenteric artery or the common hepatic artery. Japanese surgeons have tried to cure this stage of pancreatic cancer (Stage IVa of JCS version 4,[21] T3 Stage II of UICC system), which accounts for the largest number of patients, by surgery. However, despite the fact that pancreatoduodenectomy has been performed safely worldwide for the past 5 years, with an operative death rate lower than 5%,[13] few surgeons in the United States or other Western countries perform radical surgical resection for this stage of pancreatic cancer.[30]

We registered the randomization only after the eligibility of the stage had been confirmed by laparotomy, which was one of the distinctive features of our study. Previously, we reported discrepancies between the operatively confirmed staging and the preoperative imaging estimation of the extension of the main tumor and distant metastasis.[20] As our preliminary statistical analysis revealed surgery to be significantly better, for ethical reasons we stopped entering patients into the trial. Despite the small number of patients, we think that they represented a more homogeneous group of the same stage than in previous clinical trials based on staging with imaging modalities. Furthermore, the fact that the patients were followed-up for longer than 5 years resulted in a very high quality study.

This study was difficult to conduct in Japan where most patients with pancreatic cancer elect to undergo surgery, even though few survive for longer than 5 years. However, it is also true that a patient with pancreatic cancer rarely survives for longer than 3 years with nonsurgical treatments alone. According to a nationwide survey by the Japanese Pancreas Society, between 1980 and 1999, a total of 2005 patients underwent resection of surgical stage IVa ductal cancer (almost identical to the stage in this study), followed by an average 1-year survival of 49%, a 5-year survival of 10%, and a 10-year survival of 5%.[5] No data were available on the results of radiochemotherapy in comparable patients. Our randomized controlled trial revealed that radiochemotherapy administered to comparable patients resulted in significantly shorter survival than surgery. This difference between the treatment groups was greater than expected and detected with a much smaller number of patients than estimated at the time of sample size determination.

It was once thought that liver metastasis was likely to develop after radical surgery; however, the increased number of long-term survivors after radical surgery in Japan suggests that at least some patients with locally advanced pancreatic cancer have limited disease and will benefit greatly from surgical resection. The fact that this study was performed by a group of specialized institutions focusing on pancreatic diseases should be taken into account because recent reports show a distinct association between high patient volume and decreased mortality rates.[12,31–33]

Results from recent large randomized trials have shown that adjuvant therapy with fluorouracil had a significant survival benefit in patients with resected pancreatic cancer[34] and that postoperative gemcitabine delayed the development of recurrent disease after resection.[35,36] The effective combination of these adjuvant chemotherapies with curative-intent surgical resection could prolong the survival time of the patients greatly.

In conclusion, surgery is currently the best treatment for advanced pancreatic cancer without distant metastasis, extending beyond the pancreatic capsule but not invading the superior mesenteric artery or common hepatic artery. This type of surgery must be performed by an experienced surgeon at a large hospital.

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Clinical significance of plasma metastin level in pancreatic cancer patients

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Abstract. Metastin, which is a 54-residue peptide coded by *KiSS-1* gene, is an endogenous ligand to a G-protein-coupled receptor GPR54. Metastin suppresses a malignant tumor to metastasize and regulates secretion of gonadotropine releasing hormone. Physiological action of metastin has been focused on in oncology. It is reported that less *KiSS-1* gene and more hOT7T175 gene which codes GPR54 are expressed in pancreatic cancers than in normal pancreatic tissues; however, there is no study that investigates the relationship between clinicopathological characteristics and plasma metastin concentration in pancreatic cancer patients. The purpose of this study was to investigate the relationship between plasma metastin-like immunoreactive substance (LI) levels and clinical characteristics in pancreatic cancer patients. Thirty-three patients with pathologically confirmed pancreatic cancer before or just after treatments and 24 healthy volunteers were included in the study. Patients were grouped according to the International Union Against Cancer TNM classification. Plasma metastin-LI was measured by enzyme immunoassay. The plasma metastin-LI levels of cancer patients were significantly higher when compared with healthy volunteers. Significant relationship was not found between the plasma metastin-LI levels and the clinico-pathological factors such as tumor size, invasion, lymph node metastasis and distant metastasis. The plasma metastin levels may be a significant biomarker to predict the presence of pancreatic cancer and could be used in pancreatic cancer screening.

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Introduction

Metastasis is the most life-threatening complication of solid tumors. When surgical intervention achieves complete resection of the primary neoplasm, the patient can be cured unless there are micro-metastases at distant sites. Tumor metastasis is a complex multi-step process, involving invasion of primary cancer cells into local tissue, angioinvasion, cell migration, reimplantation and proliferation. In addition, previous data have demonstrated the importance of cell surface receptors in determining the likelihood and location of metastatic tumor implants (1). Therefore, it is clear that inhibition of metastasis would result in improved clinical outcome for most cancers.

Metastin has been identified as the endogenous ligand for an orphan heptahelical receptor (hOT7T175, GPR54) that couples primarily to Gp/11 (2-5). The binding of metastin to its receptor has been shown to inhibit chemotaxis *in vitro*, to enhance the expression and activity of focal adhesion kinase and to inhibit the ability of metastin receptor-overexpressing melanoma cells to metastasize *in vivo* (6).

Metastin is expressed in normal organs such as placenta, testis, liver, small intestine and pancreas (6). GPR54 is overexpressed in melanoma (2), hepatocellular (3), thyroid (4), breast (5), esophageal (7), bladder (8) and pancreatic cancer tissues (9). However, the effect of metastin differs according to the type of cancer; lack of KiSS-1 leads to poor progression of bladder cancer and poor outcome (8); lack of KiSS-1 and hOT7T175 predicts lymph node metastasis of esophageal cancer (7); KiSS-1 is overexpressed in advanced breast cancer (5); and over-expression of KiSS-1 and hOT7T175 causes progression of liver cancer (3).

In pancreatic cancer tissue, KiSS-1 is less expressed and hOT7T175 is more expressed when compared with those in normal pancreas tissue (9). However, there are no studies on the relationship between plasma metastin levels and clinical characteristics or outcome of pancreatic cancer. The purpose of this study was to investigate the relationship between plasma metastin-LI level and clinical characteristics in pancreatic cancer patients.

Patients and methods

Patients and healthy volunteers. The study design was approved by the Ethics Committees of Oita Medical University and Kyoto University Hospital. All patients and healthy volunteers included in this study received information on the design and scientific purposes of the study and gave written informed consent.

Thirty-three patients with pathologically confirmed pancreatic cancer (17 female, 16 male, aged 42-79 years) were included in the study. Blood sampling was performed in the morning, before the patients were treated by chemotherapy, radiation therapy or surgery. While surgical resection was performed for the patients with resectable cancer, chemotherapy was performed for the patients with locally advanced cancer and/or distant organ metastasis. The patients were evaluated according to the UICC (International Union Against Cancer) TNM classification 6th edition (10) based on the pathological findings.

Twenty-four healthy volunteers (12 female, 12 male) (aged 46-83 years) were included in the study. The subjects did not receive any medication 1 month before and during the study.

Materials. Synthetic human metastin-10 was purchased from the Peptide Institute (Osaka, Japan). Antiserum to metastin-10 (G-048-56) was purchased from Phoenix Pharmaceuticals (Belmont, CA, USA). Goat affinity-purified antibody to rabbit IgGs (whole molecule) (55641) was purchased from ICN Pharmaceuticals (Aurora, OH, USA). MUG and EMC-succinimide were purchased from Sigma (St. Louis, MO, USA). ß-Galactosidase and aprotinin (Trasylol) were purchased from Boehringer Mannheim (Mannheim, Germany) and Bayer (Leverkusen, Germany), respectively. All other reagents were analytical grade reagents from commercial sources.

EIA procedure for metastin-LI. Peptide levels in plasma were measured using highly sensitive EIAs for metastin-LI, that was developed by us and has been described previously (11). Assays were performed using a delayed-addition method. Separation of bound and free antigens was performed on anti-rabbit IgG-coated immunoplates (Nunc-Immuno Module Maxisorp F8; InterMed, Denmark). Human metastin-10 was conjugated with ß-D-galactosidase using EMC-succimide according to the methods previously reported (12). The EIAs for metastin-LI were specific and highly sensitive (11).

Preparation of plasma extracts. Blood samples were placed in chilled tubes containing aprotinin (500 KIU/ml) and EDTA (1.2 mg/ml) and centrifuged immediately. Each plasma aliquot was diluted 5-fold with 4% acetic acid (pH 4.0) and loaded onto a C18 reversed-phase cartridge (Sep-Pak C18; Millipore, Milford, MA, USA). After washing with 4% acetic acid, peptides in plasma 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 assayed.

Statistical analysis. The results are expressed as box and whisker plot (highest, third quartile, median, first quartile and lowest value). Comparison of the results was made by Mann-Whitney U test. $P<0.05$ indicates statistical significance.

Table I. Clinical and histological characteristics of the patients with pancreatic cancer.

Patients, n	33
Gender, n (%)	
Male	17 (51.5)
Female	16 (48.5)
Mean age (SD), years	60.9 (9.7)
TNM classification, n (%)	
T-primary tumor	
T2	3 (9.1)
T3	22 (66.7)
T4	8 (24.2)
N-regional lymph node	
NX	3 (9.1)
N0	7 (21.2)
N1	23 (69.7)
M-distant metastasis	
M0	19 (57.6)
M1	14 (42.4)
Stage, n (%)	
1B	1 (3.0)
2A	5 (15.2)
2B	12 (36.4)
3	1 (3.9)
4	14 (42.4)
Tumor invasion to, n (%)[a]	
Artery	11 (33.3)
Portal vein	16 (48.5)
Nerve	19 (57.6)
Other organs	2 (6.1)
Distant metastasis to, n (%)[b]	
Liver	8 (24.2)
Lymph node	8 (24.2)
Peritoneum	4 (12.1)
Surgery, n (%)	
Resectable	13 (39.4)
Unresectable	17 (51.5)
Neoadjuvant chemotherapy + resection	3 (9.1)

[a]Patients could have more than one site of invasion. [b]Patients could have more than one site of metastasis.

Results

A total of 33 pancreatic cancer patients and 24 healthy volunteers were included in the study (Table I). There was no significant difference in ages between pancreatic cancer patients and healthy volunteers (P=0.698).

In pancreatic cancer patients, as well as in healthy volunteers, there was no significant correlation between plasma metastin-LI levels and age (P=0.204 and 0.667)

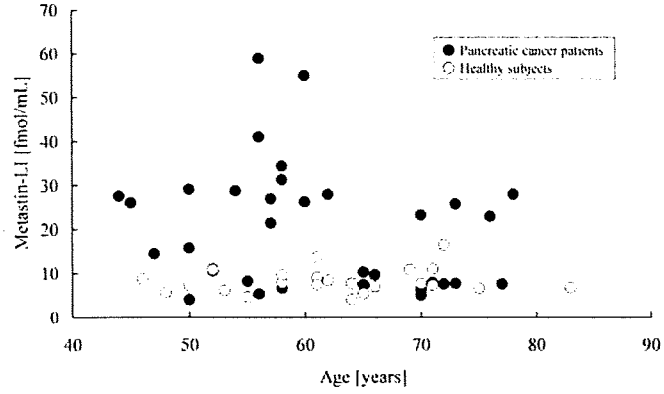

Figure 1. The relationship between age and plasma metastin-LI levels. [● Pancreatic cancer patients (n=33) and ○ healthy volunteers (n=24)].

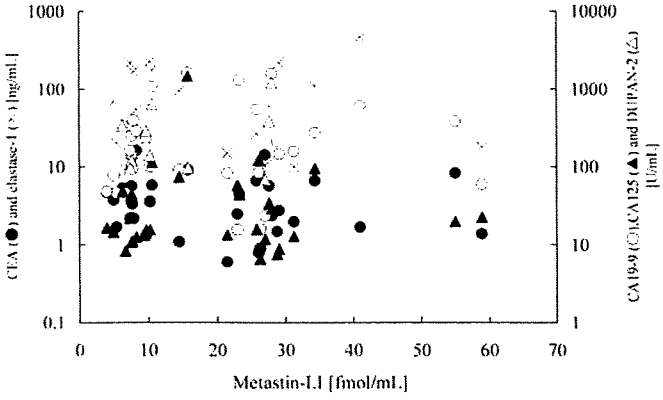

Figure 2. The relationship between some tumor markers and plasma metastin-LI levels. [● CEA (n=32), ○ CA19-9 (n=32), ▲ CA125 (n=29), △ DUPAN2 (n=11), X elastase-1 (n=25)].

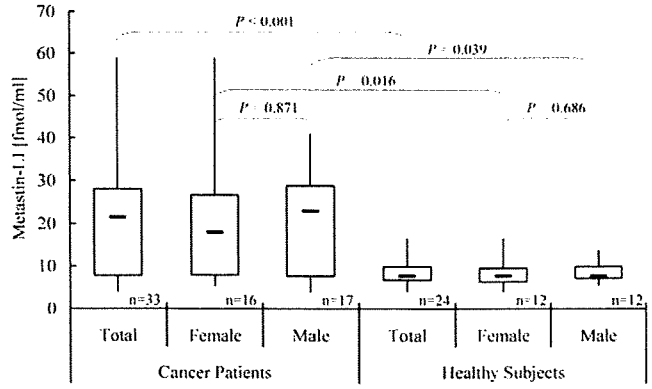

Figure 3. Comparison of plasma metastin-LI levels between pancreatic cancer patients and healthy volunteers. Each value represents the box and whisker plot (highest, third quartile, median, first quartile and lowest value). *P<0.05, significantly different compared to plasma metastin-LI levels in healthy volunteers.

(Fig. 1). The plasma metastin-LI levels also showed no significant correlation with tumor markers [carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), CA125, pancreatic cancer-associated antigen (DUPAN-2), elastase-1] (P=0.788, 0.721, 0.767, 0.155, 0.420) (Fig. 2).

There were no significant differences in the plasma metastin-LI levels between female and male in healthy volunteers and pancreatic cancer patients. The plasma metastin-LI levels in pancreatic cancer patients were significantly higher than those in healthy volunteers (Fig. 3). Although the plasma metastin-LI levels in patients with T2 tumor were not significantly different from those in healthy volunteers (P=0.855), the plasma metastin-LI levels in patients of T3, T4, N0, N1, M0 and M1 group were significantly

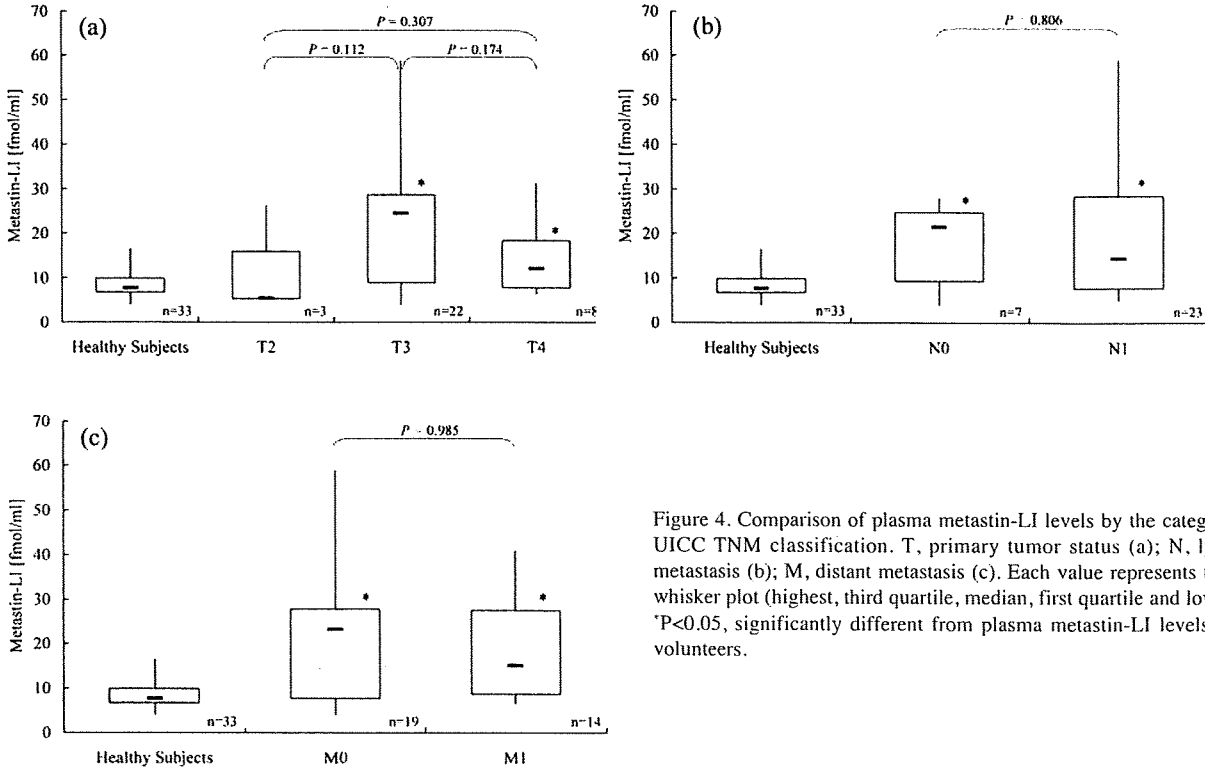

Figure 4. Comparison of plasma metastin-LI levels by the categories of the UICC TNM classification. T, primary tumor status (a); N, lymph node metastasis (b); M, distant metastasis (c). Each value represents the box and whisker plot (highest, third quartile, median, first quartile and lowest value). *P<0.05, significantly different from plasma metastin-LI levels in healthy volunteers.

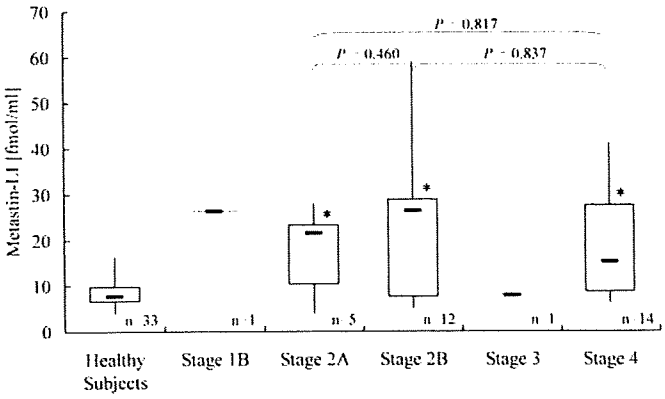

Figure 5. Comparison of plasma metastin-LI levels by the UICC stage. Each value represents the box and whisker plot (highest, third quartile, median, first quartile and lowest value). *P<0.05, significantly different from plasma metastin-LI levels in healthy volunteers.

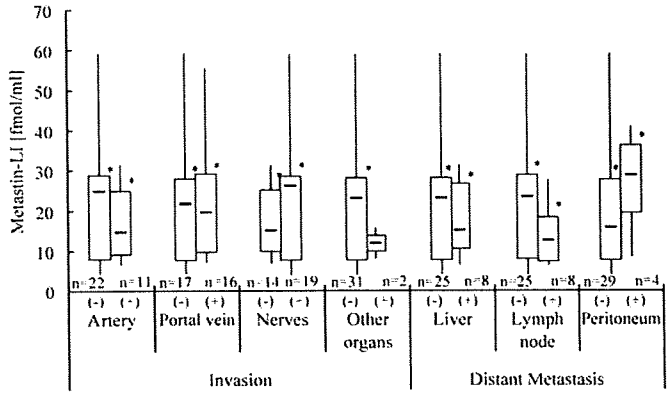

Figure 6. Comparison of plasma metastin-LI levels by the status of invasion or metastasis stage. Each value represents the box and whisker plot (highest, third quartile, median, first quartile and lowest value). *P<0.05, significantly different from plasma metastin-LI levels in healthy volunteers.

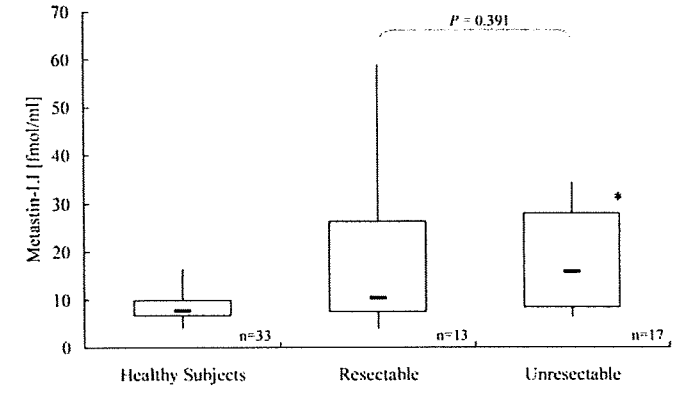

Figure 7. Comparison of plasma metastin-LI levels by the resectability. Each value represents the box and whisker plot (highest, third quartile, median, first quartile and lowest value). *P<0.05, significantly different from plasma metastin-LI levels in healthy volunteers.

higher than those in healthy volunteers (P<0.001, 0.017, 0.009, <0.001, <0.001, <0.001).

Plasma metastin-LI levels in cancer patients did not differ by categories of the UICC TNM classification (Fig. 4a-c). Plasma metastin-LI levels did not significantly differ by UICC stage (Fig. 5). The plasma metastin-LI levels in patients of stage 2A, 2B and 3 groups were significantly higher than those in healthy volunteers (P=0.027, 0.005, <0.001).

Plasma metastin-LI levels did not differ significantly by the status of invasion or metastasis [P=0.504 (artery), 0.313 (portal vain), 0.702 (nerves), 0.983 (liver), 0.179 (lymph node), 0.225 (peritoneum)] (Fig. 6).

The plasma metastin-LI levels were not significantly different between resectable and unresectable patients (Fig. 7). Although the plasma metastin-LI levels in resectable patients were not significantly different from those in healthy volunteers (P=0.166), those in unresectable patients were significantly higher than in healthy volunteers (P=0.001).

Discussion

Metastin is known to have a stimulating effect on gonadotropin secretion (13) and is related to surge secretion of GnRH in the central nervous system (14). It is reported that peripheral (plasma) metastin levels are increased by pregnancy (15). Although a relationship between central and peripheral metastin has not been established, aging or gender can change movement of central and/or peripheral metastin. In the current study, however, there was no relationship between age and plasma metastin-LI levels (Fig. 1) and no significant difference between female and male in healthy individuals and in patients (Fig. 3).

Interestingly, we first showed that the plasma metastin-LI levels in pancreatic cancer patients were significantly higher when compared to healthy subjects who were of a similar age (Fig. 3), indicating that pancreatic cancer tissue has an influence on the level of plasma metastin-LI. However, plasma metastin-LI levels did not correlate with several tumor markers in cancer patients (Fig. 2). Although we did not measure the tumor marker levels in healthy subjects, the plasma metastin-LI levels might correlate with the tumor markers if we could add the data from non-cancer subjects.

Since the pancreas is located deep inside the body, the diagnosis of cancer is made by imaging such as computerized tomography or echography, but the tumor biopsy is sometimes very difficult. Furthermore, symptoms that are recognized by the patient, such as abdominal pain, jaundice, weight loss and gastrointestinal dysfunction may not appear until the cancer has progressed greatly. If the measurement of plasma metastin level enables the prediction of presence of pancreatic cancer, it may be of great help to diagnose the disease at an early stage.

The results of the current study suggest that the plasma metastin-LI of pancreatic cancer patients possibly is not a predictive indicator for tumor progression, invasion and lymph node and distant metastases (Figs. 4 and 5). In addition, there were no significant differences in the plasma metastin-LI levels in resectable and unresectable pancreatic cancer patients, although the metastin-LI levels in unresectable patients were significantly different from healthy volunteers. Further study will be necessary to establish cut-off values to predict extra-pancreatic progression and/or resectable status of this disease.

It is important to investigate whether metastin could be a biomarker to predict recurrence of pancreatic cancer and/or efficacy of treatments.

It is known that almost all cancer tissues, as well as pancreatic cancer, do not overexpress metastin, when compared with normal tissues and metastin has a metastasis-suppressive effect (6). Although the amount of *KiSS-1* does not always reflect plasma metastin levels, it is difficult to imagine that cancer tissue secretes a substance which stops their progression. A plausible explanation for the high level of plasma metastin-LI in cancer patients is that metastin may be secreted from normal tissue as a reaction to cancer progression, which is a self-defense mechanism. There have been no studies that measured plasma metastin levels in other kind of cancer, therefore, it is not known whether all kinds of cancer show such a profile as plasma metastin levels directly proportional to cancer progression. Gender-hormone-dependent cancer, such as breast and prostate cancers, may have quite a different aspect.

Although further prospective study is needed, the measurement of plasma metastin levels and investigation of the relationship between plasma metastin and clinical cancer stage is important, not only to reveal the physiological action of metastin, but also to evaluate its effectiveness as a biomarker. Metastin could become a biomarker to predict cancer existence and its progression.

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Original Paper

Pylorus-preserving Pancreatoduodenectomy: Preoperative Pancreatic Function and Outcome

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ABSTRACT

Background/Aims: To investigate the effects of preoperative pancreatic function on gastric emptying, body weight, and quality of life after pylorus-preserving pancreatoduodenectomy.

Methodology: Thirty-one patients who underwent pylorus-preserving pancreatoduodenectomy were divided into 2 groups according to preoperative pancreatic exocrine and endocrine function (normal *vs.* abnormal). Gastric emptying, body weight, and quality of life were evaluated before surgery, 1-2 months after surgery (short term), and 6-12 months after surgery (long term).

Results: Short-term body weight was significantly decreased in comparison to preoperative body weight regardless of preoperative exocrine and endocrine pancreatic function. Body weight returned to the preoperative level by 12 months after surgery in patients with normal preoperative pancreatic function but not in patients with abnormal pancreatic function. In both groups, gastric emptying was delayed at 1-2 months after surgery and then returned to the preoperative value by 12 months. Short-term quality of life did not differ from preoperative quality of life in either group, but long-term quality of life improved to beyond the preoperative level in both groups.

Conclusions: Preoperative pancreatic function appears to significantly influence long-term body weight after pylorus-preserving pancreatoduodenectomy.

KEY WORDS: Pancreatic function; Gastric emptying; PPPD

ABBREVIATIONS: Pylorus-Preserving Pancreatoduodenectomy (PPPD); Quality Of Life (QOL); Pancreatoduodenectomy (PD); Delayed Gastric Emptying (DGE)

INTRODUCTION

Traverso and Longmire introduced pylorus-preserving pancreatoduodenectomy (PPPD) in 1978 (1), and it is now the standard surgical procedure for treatment of periampullary lesions. It is thought that PPPD prevents long-term complications such as dumping and anorexia by preserving the reservoir function of the stomach and the duodenum-derived intestinal hormones and that, in comparison to standard pancreatoduodenectomy (PD), it improves nutritional status and quality of life (QOL) (2-5). However, complications can occur after PPPD. For example, delayed gastric emptying and impaired pancreatic function can result from the resection, and nutritional status may remain insufficient. Few studies have investigated the relation between pancreatic function and gastric emptying, nutritional status, and QOL over the long term after PPPD even though pancreatic function and gastric emptying are important indicators of postoperative nutritional status and QOL. The aim of this study was to investigate the effects of preoperative pancreatic exocrine and endocrine function on gastric emptying and recovery of body weight over the long term after PPPD.

METHODOLOGY

The present study included 31 Japanese patients who underwent PPPD in the Department of Surgery and Oncology at Kyushu University Hospital January 1994 through December 2001. The group comprised 19 men and 12 women who ranged in age from 46 to 81 years, with a mean age of 63.2 years. PPPD was performed for 19 malignant and 12 benign diseases: ampullary carcinoma, n=9; bile duct carcinoma, n=6; pancreas carcinoma, n=4; intraductal papillary adenoma of the pancreas, n=6; chronic pancreatitis, n=4; serous cystadenoma of the pancreas, n=1; and chronic cholangitis, n=1. All patients were followed up, and cancer recurrence was ruled out for more than 1 year after PPPD.

Of the 31 patients, 27 underwent gastrointestinal reconstruction by the Imanaga method (6) and 4 by the Traverso method (1). The proximal duodenum was transected 2-6cm distal to the pyloric ring. The Imanaga reconstruction procedure has been described previously (7-11): end-to-end duodenojejunostomy, end-to-side pancreatojejunostomy and hepaticojejunostomy, in that order. In Traverso reconstruction, pancreatojejunostomy is performed 5cm from the closed end of the jejunum, this is followed by hepaticojejunostomy 10cm distally and end-to-side duodenojejunostomy 30cm more distally.

Before surgery, the fecal chymotrypsin level (cut-off value: 13.2 U/g) and fasting blood sugar level (cut-

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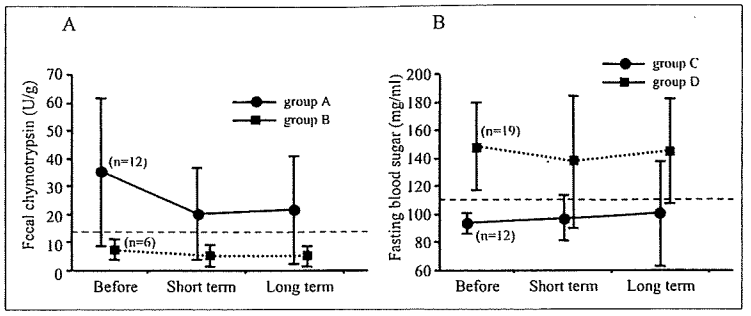

FIGURE 1 Changes in pancreatic **(A)** exocrine (fecal chymotrypsin) and **(B)** endocrine (fasting blood sugar) function.

off value: 110mg/mL) were examined in each patient for evaluation of pancreatic exocrine and endocrine function, respectively. Patients were divided into 2 groups according to preoperative pancreatic exocrine and endocrine function, and they were classified in subgroups of normal and abnormal group: group A, fecal chymotrypsin level was normal, group B, fecal chymotrypsin level was abnormal, group C, fasting blood sugar level was normal, group D, fasting blood sugar level was abnormal. Gastric emptying, body weight, and QOL were determined before surgery, 1-2 months after surgery (short term) and 6-12 months after surgery (long term). Gastric emptying was evaluated by the acetaminophen method as previously reported (7). The indices of gastric emptying were calculated from the area under the serum acetaminophen concentration curve for 90 minutes (AUC 90). Changes in each patient's body weight were calculated by referring to the preoperative level as 100%. QOL was assessed by means of a modified Kurihara questionnaire (12), which we have used previously (11,13). The questionnaire consisted of 23 items divided into 2 categories: physical (questions 1-13) and psychosocial (questions 14-23).

All values are expressed as means ± standard deviation (SD). Statistical analyses were carried out with unpaired *t*-test. A *P* value of less than 0.05 was considered significant.

RESULTS

Pancreatic Exocrine and Endocrine Function

The mean fecal chymotrypsin level in group A was decreased in the short term after surgery, but kept within normal limit in the short and long term. The level in group B with pancreatic exocrine insufficiency did not differ between time points. The mean fasting blood sugar levels in groups C and D did not differ between time points. Thus, normal or abnormal preoperative pancreatic exocrine and endocrine function did not appear to influence postoperative pancreatic function **(Figure 1A, B)**.

Influence of Preoperative Pancreatic Exocrine Function

Short-term gastric emptying was delayed in both groups (group A: before surgery, 550.8±243.9 μg·90min/mL; short term, 412.4±146.3μg· 90min/mL, and group B: before surgery, 649.8±294. 6μg·90 min/mL; short term, 478.5±348.9μg· 90min/mL). Long-term gastric emptying returned to the preoperative state in both groups (group A: long term, 702.2±284.4μg·90min/mL, and group B: long term, 761.7±345.7μg·90min/mL **(Figure 2A)**. Short-term body weight significantly decreased in both groups, while long-term body weight returned to the preoperative value in group A (short term, 90.9±4.3%; long term, 95.1±5.1%) but not in group B (short term, 89.5±5.3%; long term, 90.7±7.1%) **(Figure 2B).** Short-term QOL was decreased in group B (group A: before surgery, 70.1±16.3%; short term, 71.0±9.5%, and group B: before surgery, 71.3±15.3%; short term, 62.5±10.2%), but long-term QOL increased to greater than the preoperative level in both groups (group A: long term, 84.5±9.6%, and group B: long term, 78.4±8.2%) **(Figure 2C)**.

Influence of Preoperative Pancreatic Endocrine Function

Short-term gastric emptying was delayed in both groups (group C: before surgery, 679.1±267.0μg· 90min/mL; short term, 456.1±220.1μg·90min/mL, and group D: before surgery, 596.8±262.2μg· 90min/mL; short term, 410.4±150.8μg·90min/mL), and long-term gastric emptying returned to the preoperative level in both groups (group C: long term, 789.4±259.4μg·90min/mL, and group D: long term, 711.3±212.2μg·90min/mL) **(Figure 3A)**. Short-term body weight was decreased significantly in both groups. Whereas long-term body weight significantly recovered and returned to the preoperative value in group C (short term, 90.6±4.4%, long term,

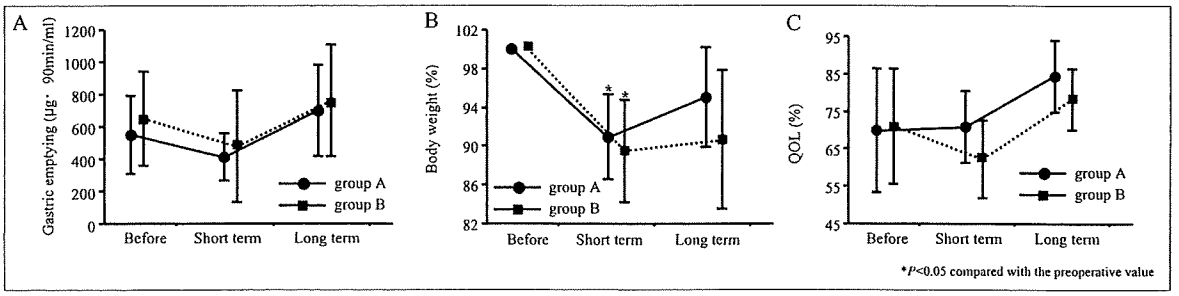

FIGURE 2 Changes in **(A)** gastric emptying (AUC 90), **(B)** body weight, and **(C)** QOL in groups A and B.

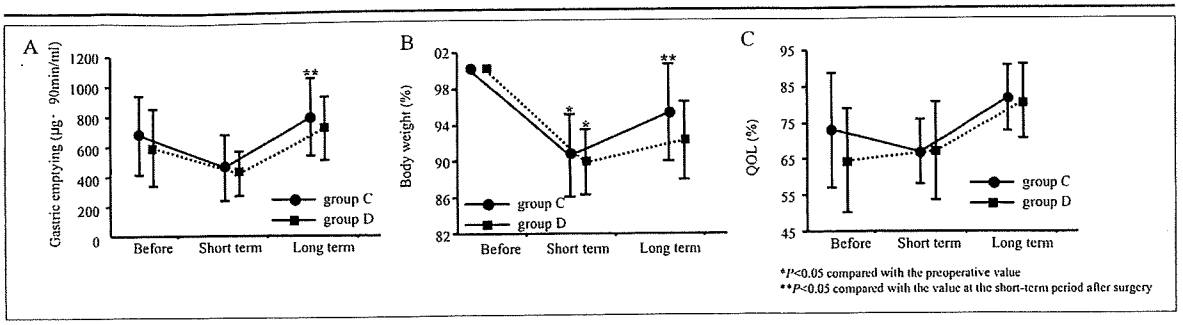

FIGURE 3 Changes in **(A)** gastric emptying (AUC 90), **(B)** body weight, and **(C)** QOL in groups C and D.

95.3±5.4%, $P<0.05$), there were no significant changes in group D (short term, 89.9±3.5%, long term, 92.2±4.2%) **(Figure 3B)**. Short-term QOL decreased in group C (before surgery, 73.0±15.8%, short term, 67.0±8.9%) and was similar in group D (before surgery, 64.5±14.4%, short term, 67.1± 13.5%). However, long-term QOL increased to greater than the preoperative level without a significant difference in both groups (group C: long term, 81.9±9.0%, and group D: long term, 80.9±10.3%) **(Figure 3C)**.

DISCUSSION

Several reports have suggested that body weight is better after PPPD than after standard PD. Kozuschek *et al.* reported that 43% of patients who underwent standard PD reached preoperative body weight after 1 year, whereas 86% of patients who underwent PPPD reached their preoperative weight (2). Braasch *et al.* reported that 28 patients who underwent PPPD reached 93% pre-illness weight and 106% preoperative weight at the time of follow-up (14). Zerbi *et al.* reported that patients who underwent PPPD had reached a mean 92% of the usual pre-illness body weight at 6 months after surgery, showing significantly better recovery of body weight than that of patients after standard PD (15). Yamaguchi *et al.* reported that postoperative loss of more than 3kg body weight was evident in 62% of patients after PPPD, that the maximum body weight loss was seen about 4.2 months after PPPD, and that body weight returned to the preoperative level 4.8 months thereafter (16). In our PPPD patients, long-term body weight was greater than 90% in all groups, and these results resembled those of previous reports. Short-term body weight decreased significantly after PPPD in patients with normal preoperative pancreatic function and in patients with abnormal preoperative pancreatic function. However, long-term body weight returned to the preoperative level in patients with normal preoperative pancreatic function, but not in patients with abnormal preoperative pancreatic function. Recovery of body weight is an important determinant of nutritional status, and our results suggest that the relation between pancreatic function and body weight is also important.

Gastric emptying is also an important determinant of nutritional status. Early delayed gastric emptying (DGE) is one of the most relevant and frequent postoperative complications and has been reported to range between 20% and 50% (17-23). The cause of DGE is not yet clear, and several factors are thought to play a role in DGE. These include gastric dysmotility after PPPD attributed to disruption of the gastroduodenal neural connection (24) and gastric dysrhythmia due to postoperative complications such as anastomotic leakage, intraabdominal abscess, and bleeding (25,26). Murakami *et al.* reported that residual pancreatic fibrosis is the most important cause of DGE after PPPD without complications (27). We previously reported that gastric emptying was delayed but returned to the preoperative level by 6 months after surgery (7). We obtained similar results in the present study, and there was no significant difference in postoperative gastric emptying between patients with normal and abnormal preoperative pancreatic function. Long-term gastric emptying was restored to the preoperative level, whereas recovery of body weight was poor in patients with abnormal preoperative pancreatic function. This suggests that preoperative pancreatic function is more important determinant of postoperative nutritional status.

Short-term QOL was the same or slightly lower than preoperative QOL. However, long-term QOL was high in comparison to preoperative and short-term QOL. It is likely that the QOL score improved with the increases in food intake and nutritional status. It is also possible that patients' anxiety over their disease state was relieved after surgery, positively influencing QOL. The difference in long-term QOL between our patients with normal and abnormal preoperative pancreatic functions suggests on indirect link between preoperative pancreatic function and improvement in QOL.

In conclusion, preoperative pancreatic function influenced the recovery of body weight after PPPD, however, it did not influence the recovery of gastric emptying or QOL.

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Journal of HBP Surgery

Contribution of ^{18}F-fluorodeoxyglucose positron emission tomography to the diagnosis of early pancreatic carcinoma

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Abstract
Background/Purpose. Pancreatic carcinoma has a poor prognosis, and early detection is essential to allow potentially curative resection. Despite the wide array of diagnostic tools available, the detection of small pancreatic tumors remains difficult. The aim of this study was to investigate the contribution of ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) to the diagnosis of early pancreatic cancer.
Methods. FDG-PET was performed in 56 patients with pancreatic cancer who underwent curative surgery. The standardized uptake value (SUV) for FDG was calculated in each patient and the relationships between the SUV and various clinicopathological factors were analyzed.
Results. The tumors ranged from 0.8 to 6.5 cm in diameter. When the cutoff value for the SUV was set at 2.5, 51 of the 56 patients (91%) had a positive FDG-PET study. The SUV did not show a significant difference in relation to tumor differentiation or pTS and pT factors. There was also no correlation between the SUV and the maximum tumor diameter ($r = 0.22$; $P = 0.1$). Five tumors had an SUV below the cutoff value, and all of these lesions had intermediate or scirrhous stroma rather than medullary stroma.
Conclusions. These results indicate that FDG-PET is useful for the detection of small early pancreatic cancers.

Key words Invasive ductal carcinoma of the pancreas · 18 F-Fluorodeoxyglucose · positron emission tomography · Diagnostic imaging

Introduction

Pancreatic cancer is one of the leading causes of cancer death. This tumor currently kills more than 20000 persons per year in Japan, and is the fifth highest cause of death from cancer.[1–4] The results of surgical treatment are poor, including radical resection with curative intent.[5–7] In fact, the overall 5-year survival rate of patients with margin-negative resection is reported to be only 6.8%–25%.[8–13]

There is no clear definition of early pancreatic carcinoma. According to the TNM staging system of the International Union Against Cancer (UICC), tumors confined to the pancreas with a diameter of less than 2 cm are T1 and are classified as stage IA. Similarly, the classification system of the Japan Pancreas Society (JPS)[14] states that tumors confined to the pancreas with a diameter of less than 2 cm are T1 and are classified as stage I. Therefore, in the present study, we defined early pancreatic carcinoma as a tumor with a diameter of less than 2 cm and we investigated whether small cancers could be diagnosed by current imaging modalities.

Patients with early pancreatic carcinoma have no typical signs or symptoms, which makes it very difficult to detect and diagnose this cancer at an early stage. Because conventional imaging methods are relatively ineffective at identifying small and potentially curable pancreatic carcinomas, patients can miss the chance of obtaining surgical cure. Sensitive and specific imaging modalities are thus needed to improve the diagnosis of early pancreatic cancer.

The increased uptake of fluorine-18 fluorodeoxyglucose (^{18}F-FDG) due to the enhanced glucose metabolism of cancer cells is a sensitive marker of tumor viability. The detection of increased ^{18}F-FDG uptake by positron emission tomography (PET) has been used in diagnostic imaging for several types of hepatobiliary[15–17] and pancreatic cancers,[18–21] and studies have shown that FDG-PET is useful for the characterization of pancreatic tumors, as well as for the assessment of the therapeutic response and outcome.[22,23] However, the relationship between tumor size and FDG uptake has not yet been examined for pancreatic cancer. Accordingly, this study was performed to assess the contri-

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bution of FDG-PET to the diagnosis of small early pancreatic cancers.

Patients and methods

Study population

Patients with histologically confirmed invasive ductal carcinoma of the pancreas who had no clinical, radiographic, or intraoperative evidence of distant metastasis were reviewed, revealing a series of 56 patients who underwent surgery at Kyoto University Hospital between March 2004 and February 2007. Tumor size was determined from examination of the resected specimens. Staging of the disease was performed according to the classification of the JPS[14] in order to use its fine definition of the tumor size.

PET study

^{18}F was produced by a ^{20}Ne (d, alpha) ^{18}F nuclear reaction, and ^{18}F-FDG was synthesized by nucleophilic substitution using an F-100 ^{18}F-FDG synthesizer (Sumitomo Heavy Industries, Tokyo, Japan) and a CYPRIS-325R cyclotron (Sumitomo Heavy Industries). All patients were examined by using a high-resolution, whole-body PET scanner with an 18-ring detector array (Advance; General Electric Medical Systems, Milwaukee, WI, USA).

Patients fasted for at least 4 h before receiving an intravenous injection of ^{18}F-FDG (296 ± 74 MBq), and the acquisition of whole-body PET images was started 50 min after the injection. Each patient lay supine on the PET table with his/her arms at the side of the body, and was held in place by a belt around the abdomen. Data acquisition (emission and transmission scans) was performed in the two-dimensional imaging mode with septae in place. Emission images were acquired for 3 min per bed position and each post-emission transmission scan was obtained for 1 min per position. A whole-body scan (from the face to the upper thighs) was performed in each patient, using five or six bed positions according to the patient's height. Data were reconstructed by the ordered subsets expectation maximization (OSEM) method, using 16 subsets, 3 iterations, and a 128 × 128 array.

Image analysis

PET images were interpreted by at least two experienced nuclear medicine physicians, using all available clinical information and correlative conventional imaging for anatomic guidance. For semiquantitative analysis of ^{18}F-FDG uptake, regions of interest (ROIs) were manually defined on transaxial tomograms. If no region of high uptake was detectable by PET, the ROI was drawn from the findings on abdominal computed tomography (CT). The maximum standardized uptake value (SUV) was calculated as follows for quantitative analysis of tumor ^{18}F-FDG uptake:

$$\text{SUV} = \text{C (kBq/ml)}/[\text{ID (kBq)} \times \text{body weight (kg)}]$$

where C represents the tissue activity concentration measured by PET, and ID represents the injected dose.

Statistical analysis

Values for results are expressed as means ± SD. Differences between two groups were analyzed using the Mann-Whitney *U*-test (StatView PowerPC version; SAS Institute, Cary, NC, USA) and $P < 0.05$ was considered statistically significant.

Results

Table 1 shows the clinicopathological profiles of the 56 patients. In all patients, the diagnosis of pancreatic adenocarcinoma was histologically confirmed. The tumor was well differentiated in 14 patients, moderately differentiated in 38 patients, and poorly differentiated in 4 patients. Tumor diameter ranged from 0.8 to 6.5 cm. Based on a cutoff value of 2.5 for the SUV, 51 of the 56 patients (91%) had a positive FDG-PET study.

For well-differentiated, moderately differentiated, and poorly differentiated tumors, the SUVs were 5.3 ± 2.5 (n = 14), 5.5 ± 2.4 (n = 38), and 6.5 ± 3.2 (n = 4), respectively. There were no significant differences in SUV in relation to tumor differentiation (Fig. 1).

Table 2 shows the sensitivity and SUV of each pathological tumor size (pTS) factor. The sensitivity of PET for detecting pTS1, pTS2, and pTS3 tumors was 81.3%, 93.9%, and 100%, respectively. Although the sensitivity was higher for larger tumors, the SUV was not significantly associated with the pTS factor. In addition, there was no correlation between SUV and the maximum tumor diameter (r = 0.22; P = 0.1; Fig. 2).

Table 3 shows the sensitivity and SUV for each tumor invasion (pT) factor. The sensitivity of PET was 50% for pT1 tumors, 100% for pT2 tumors, 95% for pT3 tumors, and 96.9% for pT4 tumors. Thus, its sensitivity for pT1 tumors was low, but only two patients had such tumors. The SUV was not significantly associated with the pT factor.

Only six patients had symptoms at the time of diagnosis of pancreatic cancer. The SUV was 2.5 ± 0.5 for asymptomatic patients versus 5.5 ± 1.3 for symptomatic patients, showing a significant difference between the

Table 1. Clinicopathological profiles of the patients

Age (years, mean ± SD [range])	65 ± 9 [42–85]
Sex (male:female)	29:27
Tumor differentiation	
Well-differentiated	14 (25%)
Moderately differentiated	38 (68%)
Poorly differentiated	4 (7%)
Maximum tumor diameter (cm, mean ± SD [range])	2.9 ± 1.2 [0.8–6.5]
SUV (mean ± SD [range])	5.5 ± 2.5 [2.3–12.2]
Operation	
Pancreatoduodenectomy	6
Pylorus-preserving pancreatoduodenectomy	28
Distal pancreatectomy	17
Total pancreatectomy	5
pTS	
pTS1	16
pTS2	33
pTS3	5
pTS4	2
pT	
pT1	2
pT2	2
pT3	20
pT4	32
pStage	
I	1
II	1
III	17
IVa	27
IVb	10

pTS, pathological tumor size factor in the Japan Pancreas Society classification system; pT, pathological tumor invasion factor in the Japan Pancreas Society classification system; pStage, pathological stage in the Japan Pancreas Society classification system; SUV, standardized uptake value

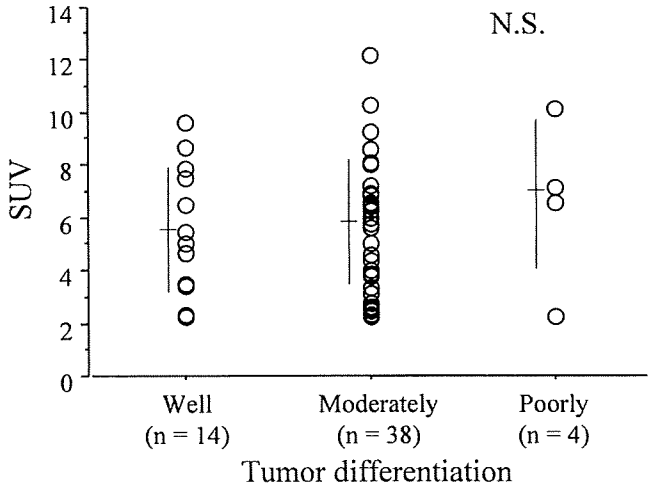

Fig. 1. Standardized uptake value (*SUV*) in relation to tumor differentiation. Well-differentiated, moderately differentiated, and poorly differentiated tumors had SUVs of 5.3 ± 2.5 (n = 14), 5.5 ± 2.4 (n = 38), and 6.5 ± 3.2 (n = 4), respectively. There were no significant differences in SUV related to tumor differentiation. *N.S.*, Not significant

Table 2. Correlations among tumor size, sensitivity, and SUV

pTS (cm)	Sensitivity (%)	SUV (mean ± SD)
pTS1 (≤2)	13/16 (81.3)	5.0 ± 2.6
pTS2 (2–4)	31/33 (93.9)	5.6 ± 2.6
pTS3 (4–6)	5/5 (100)	5.8 ± 2.0

pTS, pathological tumor size factor in the Japan Pancreas Society classification system; SUV, standardized uptake value

Table 3. Correlations among tumor invasion, sensitivity, and SUV

pT factor	Sensitivity (%)	SUV (mean ± SD)
pT1	1/2 (50.0)	2.9 ± 0.9
pT2	2/2 (100)	2.7 ± 0.6
pT3	19/20 (95.0)	5.5 ± 2.7
pT4	31/32 (96.9)	5.8 ± 2.3

pT, pathological tumor invasion factor in the Japan Pancreas Society classification system; SUV, standardized uptake value

Table 4. Characteristics of FDG-PET-negative tumors

Patient no.	SUV	Tumor differentiation	Tumor stroma	Maximum diameter (cm)	pTS	pT	pStage
1	2.3	Moderately differentiated	Intermediate	1.8	pTS1	pT4	IVa
2	2.2	Moderately differentiated	Scirrhous	2.7	pTS2	pT3	III
3	2.3	Well-differentiated	Intermediate	1.6	pTS1	pT1	I
4	2.3	Well-diferentiated	Intermediate	2.5	pTS2	pT3	IVa
5	2.3	Well-diferentiated	Intermediate	1.3	pTS1	pT3	III

pTS, pathological tumor size factor in the Japan Pancreas Society classification system; pT, pathological tumor invasion factor in the Japan Pancreas Society classification system; pStage, pathological stage in the Japan Pancreas Society classification system; SUV, standardized uptake value

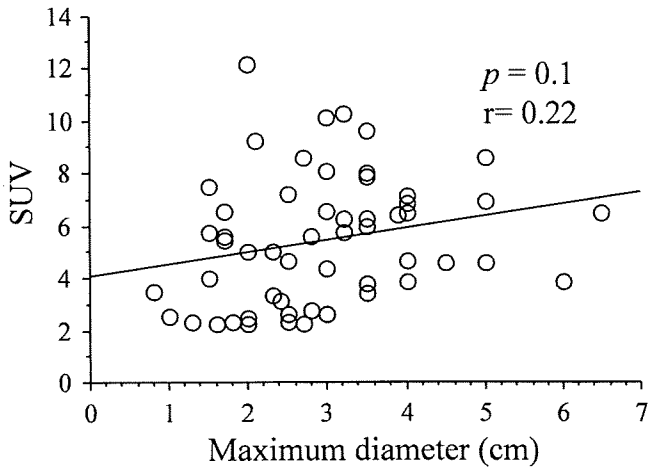

Fig. 2. Relationship between SUV and maximum tumor diameter. There was no correlation between the SUV and the maximum diameter of the tumors ($r = 0.22$; $P = 0.1$)

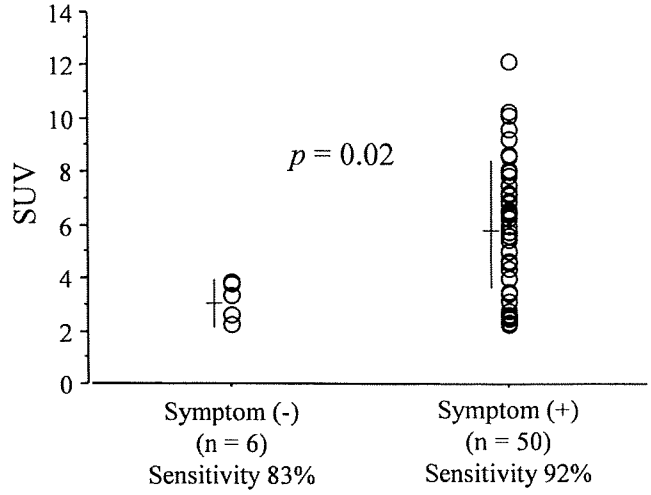

Fig. 3. Relationship between SUV and symptoms. The SUV was 2.5 ± 0.5 in asymptomatic patients and 5.5 ± 1.3 in symptomatic patients, showing a significant difference between these two groups ($P = 0.02$). However, the sensitivity of ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) was not significantly different between patients without symptoms (83%) and patients with symptoms (93%; $P = 0.48$; χ^2 test)

two groups ($P = 0.02$). However, the sensitivity of PET was not significantly different between the group without symptoms (83%) and that with symptoms (93%; $P = 0.48$; χ^2 test).

In this series, five patients had an SUV below the cutoff value of 2.5, and were judged to have a negative FDG-PET study. The characteristics of their tumors are summarized in Table 4. Four tumors had intermediate stroma and one had scirrhous stroma. Interestingly, there were no medullary tumors. The diameter of these tumors ranged from 13 to 27 mm.

Discussion

Surgical resection is the only potentially curative therapy for pancreatic cancer. Unfortunately, the presentation of this disease is usually late, so only 15%–20% of patients are candidates for pancreatectomy with curative intent.[24,25] The prognosis of pancreatic cancer is poor even in patients with potentially resectable disease, although there is some evidence that the outcome is improving slightly.[26] Early detection of small tumors is essential to allow the performance of potentially curative resection. However, the diagnosis of small pancreatic carcinomas remains difficult, even with the wide array of modalities available, such as abdominal ultrasound (US), CT, endoscopic retrograde cholangiopancreatography (ERCP), magnetic resonance imaging (MRI), and endoscopic ultrasound (EUS).

For example, the sensitivity of dual-phase contrast-enhanced helical CT in the detection of pancreatic carcinoma has been reported to vary from 76% to 92%.[27–30] However, the reported detection of small tumors has not been good enough, with a sensitivity of 67% for tumors smaller than 1.5 cm in one study,[29] 58% for tumors smaller than 2 cm in another study,[28] and 63% for tumors smaller than 2 cm in another study.[31] More recent studies have shown a detection sensitivity of 81%

for all resectable pancreatic cancer, whereas the detection sensitivity was 53% for tumors 2.5 cm or smaller in one study,[32] and 77% for tumors 2 cm or smaller in another study.[33] The sensitivity of EUS or MRI has been reported to be the same or slightly better when compared to that of CT; however, these would not be the first-line modalities.[32,34]

FDG-PET is a well-established diagnostic tool for the detection of malignant tumors,[35] and for monitoring the response to treatment of several cancers.[36,37] FDG-PET has been reported to show a sensitivity of 82% to 100% and a specificity of 67% to 100% for the diagnosis of pancreatic carcinoma,[38–42] and it has a low false-positive rate, of only 2.6%.[39,40] In the present study, FDG-PET showed positive uptake in 51 of 56 patients with pancreatic cancer (91%) when the cutoff value for the SUV was set at 2.5.

We previously reported that the SUV was significantly higher for poorly differentiated hepatocellular carcinomas (HCCs) than for well-differentiated or moderately differentiated HCCs.[15] However, the present study showed that the SUV of pancreatic cancer did not differ significantly in relation to tumor differentiation. The SUV was also not related to the pTS factor (tumor size). In addition, there was no correlation between the SUV and the maximum tumor diameter. In our series, we had 16 tumors smaller than 2 cm, and the sensitivity of FDG-PET for the detection of these tumors was 81.3%, which is still not good enough. However, when a small tumor can be detected by FDG-PET we can realize that there is a malignant tumor in the pancreas at the same time, which is unique and important information in addition to other imaging modalities. To our knowledge, this is the first study that has focused on FDG uptake by small early pancreatic cancers.

The accumulation of FDG is presumed to occur due to enhanced glucose utilization by tumor cells, which is based on the observation that cancer cells are characterized by an increase in glucose metabolism compared with healthy cells.[43] We have previously reported a close relationship between FDG accumulation during PET and the expression of glucose transporter-1.[44] However, other factors could also have an influence on FDG uptake. We speculate that tumor cellularity is one of the important factors. Scirrhous tumors have low cellularity, and thus should show less accumulation of FDG. In the present series, there were five tumors with an SUV below the cutoff value. These tumors were all of the scirrhous or intermediate type, and there were no medullary tumors, supporting our speculation.

It has been reported that small early pancreatic cancers are not associated with any typical signs or symptoms. In the present study, the majority of the patients had stage III or IV disease; 50 of the 56 patients had symptoms at the time of diagnosis and only 6 patients were asymptomatic. We found that the sensitivity of FDG-PET for asymptomatic tumors was 83% and this was not significantly different from the sensitivity for symptomatic tumors, indicating that FDG-PET studies may be useful even in patients without symptoms. Because the prognosis of pancreatic cancer is highly dependent on its stage, a simple and reliable screening method could be very useful, especially for high-risk populations, and FDG-PET could be a possible screening method for pancreatic cancer.

Most previous studies of PET in relation to pancreatic disease have focused on the differentiation between pancreatic cancer and chronic pancreatitis.[20,21] However, we showed that FDG-PET is useful not only for the differentiation between pancreatic cancer and chronic pancreatitis but also for the detection of small early pancreatic tumors.

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Midkine promoter-based conditionally replicative adenovirus therapy for midkine-expressing human pancreatic cancer

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Abstract

Background: To develop a novel therapeutic strategy for human pancreatic cancer using a midkine promoter-based conditionally replicating adenovirus.

Methods: We examined midkine mRNA expression and midkine protein expression by seven human pancreatic cancer cell lines (AsPC-1, BxPC-3, CFPAC-1, HPAC, MIAPaCa-2, PANC-1, and Suit-2), as well as by non-cancerous pancreatic tissue and pancreatic cancers. Midkine promoter activity was measured in cancer cell lines by the dual luciferase reporter assay. Adenoviral transduction efficiency was assessed by fluorescent staining of cancer cell lines using adenovirus type 5 containing the green fluorescent protein gene (Ad5GFP). Replication of adenovirus type 5 containing the 0.6 kb midkne promoter (Ad5MK) was assessed by the detection of E1 protein in cancer cell lines. The cytotoxicity of Ad5MK for cancer cells was evaluated from the extent of growth inhibition after viral infection. Infection and replication were also assessed in nude mice with subcutaneous Suit-2 tumors by intratumoral injection of Ad5MK, Ad5GFP, or vehicle. E1a mRNA expression in the treated tumors and expression of the replication-specific adenoviral hexon protein were evaluated. Finally, the anti-tumor activity of Ad5MK against intraperitoneal xenografts of Suit-2 pancreatic cancer cells was examined after intraperitoneal injection of the virus.

Results: Both midkine mRNA expression and midkine protein expression were strong in AsPC-1 and CFPAC-1 cell liens, moderate in BxPC-3, HPAC, and Suit-2 cell lines, and weak in PANC-1 and MIAPaCa-2 cell lines. Expression of midkine mRNA was significantly stronger in pancreatic cancers than in non-cancerous pancreatic tissues. The relative luciferase activity mediated by the 0.6 kb midkne fragment in AsPC-1, PANC-1, and Suit-2 cell lines was approximately 6 to 20 times greater than that in midkne-negative MIAPaCa-2 cell lines. Pancreatic cancer cell lines exhibited a

heterogeneous adenoviral transduction profile. E1A expression was higher in cell lines with strong midkine expression than in cell lines with weak midkine expression. Ad5MK showed much greater cytotoxicity for midkine-expressing Suit-2 and PANC-1 cell lines than for midkine-negative MIAPaCa-2 cell lines. In the Suit-2 subcutaneous xenograft model, expression of E1A was detected in Ad5MK-treated tumors, but not in untreated and Ad5GFP-treated tumors. In the Suit-2 intraperitoneal xenograft model, the Ad5MK group survived for significantly longer than the Ad5GFP, PBS, and untreated groups.

Conclusion: Ad5MK has an anti-tumor effect against human pancreatic cancer cell lines that express midkine mRNA. Midkine promoter-based conditionally replicative adenovirus might be a promising new gene therapy for pancreatic cancer.

Background

Pancreatic cancer is one of the most lethal malignant tumors, and it was estimated that approximately 200,000 people died of this cancer worldwide in the year 2000 [1]. It is the fifth leading cause of cancer death in Japan and the fourth in the United States [2]. Unfortunately, the pancreas is located at an inaccessible site within the abdomen, making the diagnosis of pancreatic cancer more difficult than that of other digestive tract cancers. Therefore, most patients with pancreatic cancer are diagnosed late after progression of their disease. Furthermore, pancreatic cancer frequently infiltrates neighboring tissues or vessels at an early stage, leading to a poor prognosis.

In the United States, the 1-year and 5-year survival rates of patients with pancreatic cancer are less than 25% and 5%, respectively [3]. At present, surgical resection provides the only chance of cure for these patients, but it has been reported that about 90% of patients do not undergo pancreatic resection and 58% are only given palliative treatment [4]. Moreover, recurrence after surgical resection is very common. We do not have any effective nonsurgical treatments for pancreatic cancer, because it shows strong resistance to the currently available chemotherapy and radiotherapy protocols [5]. In order to improve the clinical outcome, new modalities for the treatment of this disease are required.

Gene therapy using oncolytic viruses is one of the approaches that should be considered. The strategy of this therapy is to exploits the lytic property of virus replication to kill tumor cells. Recent knowledge of molecular biology makes it possible to modify viruses to target specific molecules or signal transduction pathways in cancer cells. Oncolytic viruses which are developed to be able to infect and replicate selectively in malignant tumor cells can spread and destroy malignant tumors without adverse effects in normal tissues.

To achieve tumor-selective viral replication, one approach has been the replacement of endogenous viral sequences with a tissue- or tumor-specific promoter. A number of tumor promoter genes such as α-fetoprotein [6], carcinoembryonic antigen [7], erbB-2 [8]and prostate-specific antigen [9,10] have been used to restrict the expression of suicide genes both *in vitro* and *in vivo*.

Midkine is a heparin-binding growth factor that is induced by retinoic acid in embryonal carcinoma cells, and it may be another candidate for this purpose [11]. The biological roles of midkine are diverse and it is closely linked to neural development [12,13] as well as to the pathogenesis of neurodegenerative diseases. At the same time, midkine is involved in the development of cancer because of its mitogenic effect [14], promotion of angiogenesis [15], anti-apoptotic activity [16], fibrinolytic activity [17], and transforming activity [18].

Midkine expression is increased in a number of malignant tumors, including esophageal, stomach, colon, hepatocellular, breast and pancreatic carcinoma, when compared with the level of expression in adjacent non-cancerous tissues [19-22]. In contrast, the expression of midkine in normal human tissues is quite limited, with moderate expression in the kidneys and weak expression in the lungs, colon, and thyroid gland [19,20,23].

On this basis, the midkine promoter could be a potential candidate for use in suicide gene therapy. Here, we demonstrate that an adenovirus vector encoding the essential adenoviral E1A gene under the control of 0.6 kb midkine promoter showed specific replication in midkine-expressing pancreatic cancer cell lines and not in non-midkine-expressing cells, and that Ad5MK selectively prevented tumor growth both *in vitro* and *in vivo*.

Methods

Cell culture and tumor samples

Seven human pancreatic cancer cell lines were used. AsPC-1, BxPC-3, CFPAC-1, HPAC, MIAPaCa-2, and PANC-1 cells were obtained from the American Type Culture Collection (Rockville, MD), and were maintained in the medium recommended by the ATCC at 37°C in a humidified atmosphere of 5% CO_2. Suit-2 cells were kindly pro-