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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

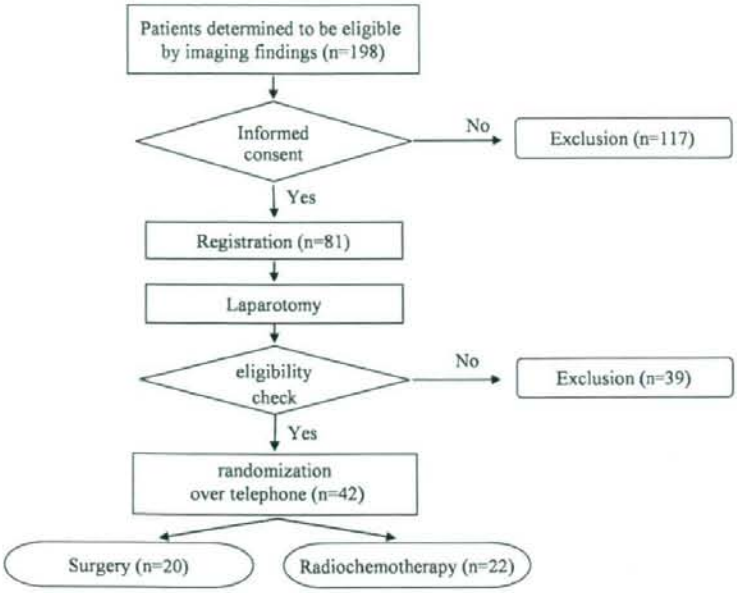

Fig. 1. Schema for the study protocol

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

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

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

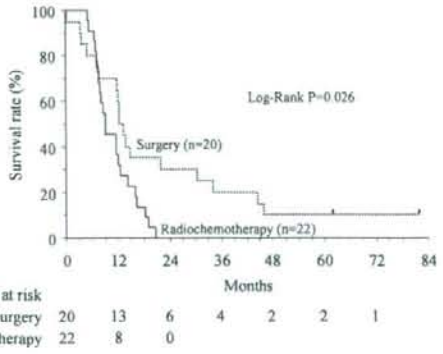

Fig. 2. Survival curves for patients treated with curative-intent surgery and those treated with radiochemotherapy. All patients were followed up for 5 years or longer

Table 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 clinicopathological 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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Key words: metastin, pancreatic neoplasm, metastasis

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)

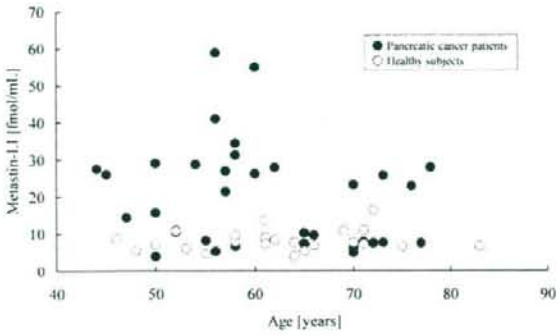

Figure 1. The relationship between age and plasma metastin-LI levels. [● Pancreatic cancer patients (n=33) and ○ healthy volunteers (n=24)].

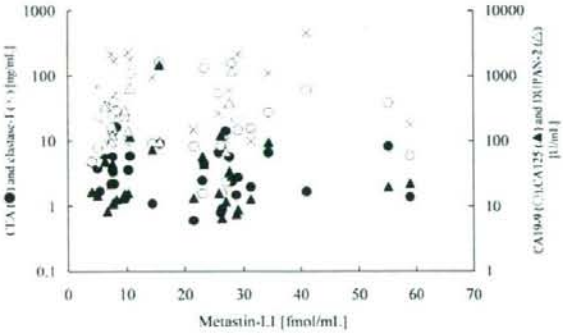

Figure 2. The relationship between some tumor markers and plasma metastin-LI levels. [● CEA (n=32), ○ CA19-9 (n=32), ▲ CA125 (n=29), △ DUPAN2 (n=11), X elastase-1 (n=25)].

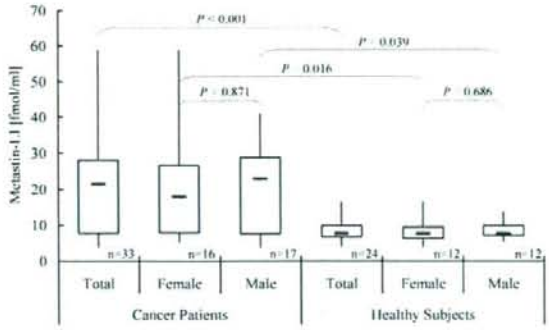

Figure 3. Comparison of plasma metastin-LI levels between pancreatic cancer patients and healthy volunteers. Each value represents the box and whisker plot (highest, third quartile, median, first quartile and lowest value). *P<0.05, significantly different compared to plasma metastin-LI levels in healthy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

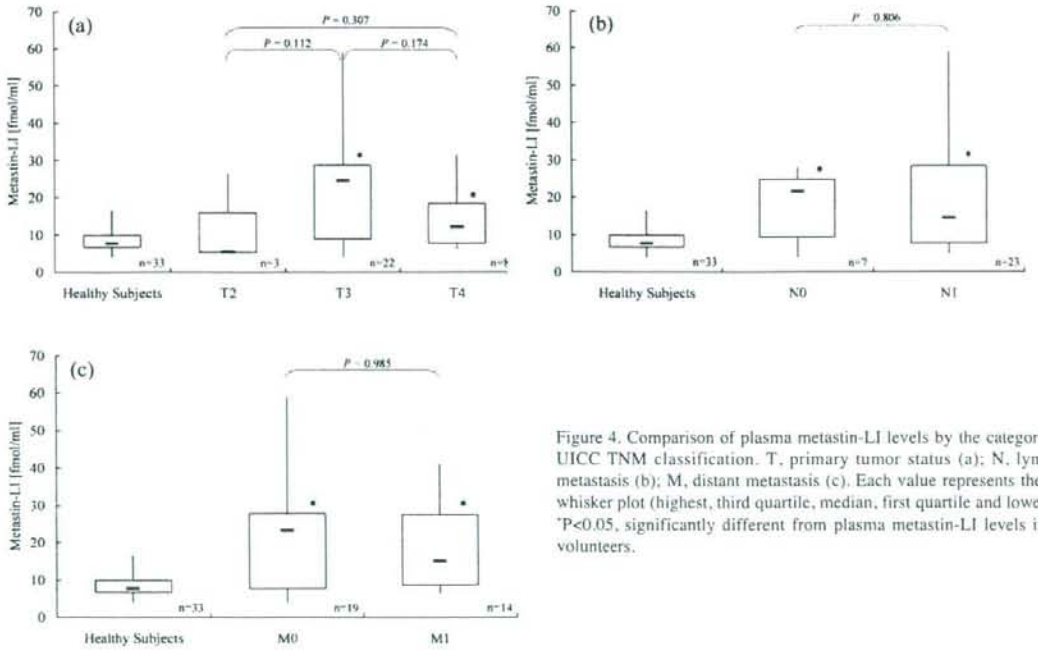

Figure 4. Comparison of plasma metastin-LI levels by the categories of the UICC TNM classification. T, primary tumor status (a); N, lymph node metastasis (b); M, distant metastasis (c). Each value represents the box and whisker plot (highest, third quartile, median, first quartile and lowest value).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

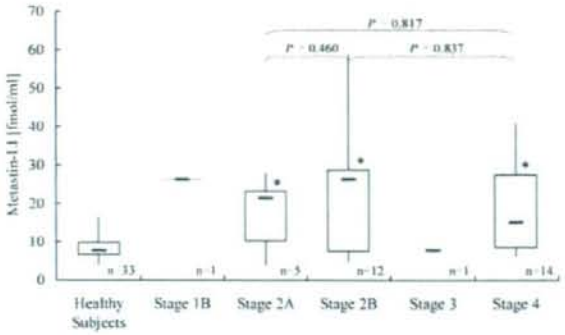

Figure 5. Comparison of plasma metastin-LI levels by the UICC stage. Each value represents the box and whisker plot (highest, third quartile, median, first quartile and lowest value). *P<0.05, significantly different from plasma metastin-LI levels in healthy volunteers.

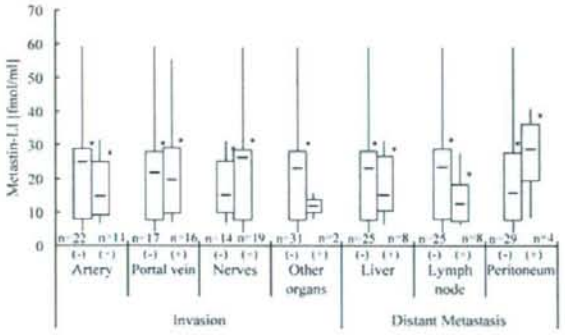

Figure 6. Comparison of plasma metastin-LI levels by the status of invasion or metastasis stage. Each value represents the box and whisker plot (highest, third quartile, median, first quartile and lowest value). *P<0.05, significantly different from plasma metastin-LI levels in healthy volunteers.

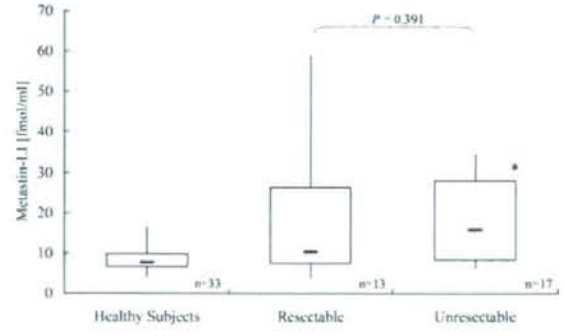

Figure 7. Comparison of plasma metastin-LI levels by the resectability. Each value represents the box and whisker plot (highest, third quartile, median, first quartile and lowest value). *P<0.05, significantly different from plasma metastin-LI levels in healthy volunteers.

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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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

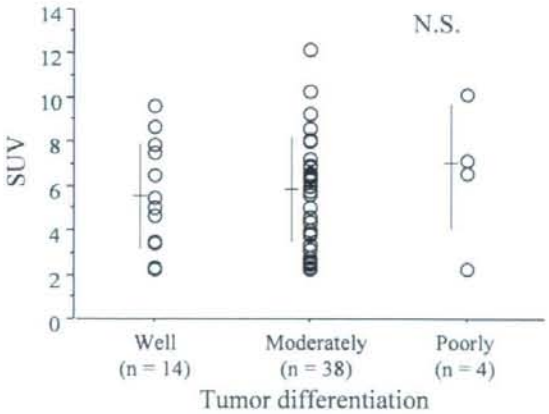

Fig. 1. Standardized uptake value (*SUV*) in relation to tumor differentiation. Well-differentiated, moderately differentiated, and poorly differentiated tumors had SUVs of 5.3 ± 2.5 (n = 14), 5.5 ± 2.4 (n = 38), and 6.5 ± 3.2 (n = 4), respectively. There were no significant differences in SUV related 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

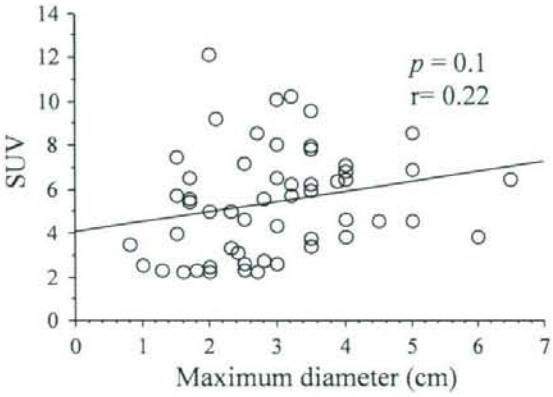

Fig. 2. Relationship between SUV and maximum tumor diameter. There was no correlation between the SUV and the maximum diameter of the tumors ($r = 0.22$; $P = 0.1$)

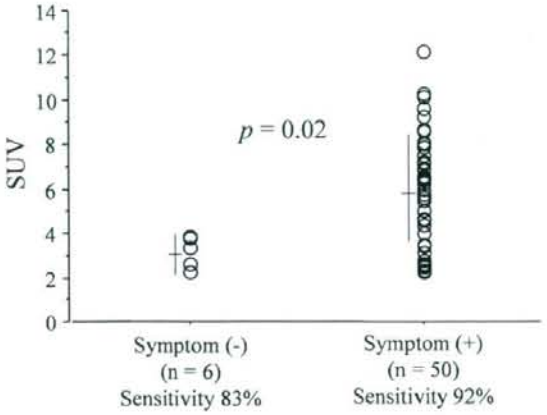

Fig. 3. Relationship between SUV and symptoms. The SUV was 2.5 ± 0.5 in asymptomatic patients and 5.5 ± 1.3 in symptomatic patients, showing a significant difference between these two groups ($P = 0.02$). However, the sensitivity of ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) was 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