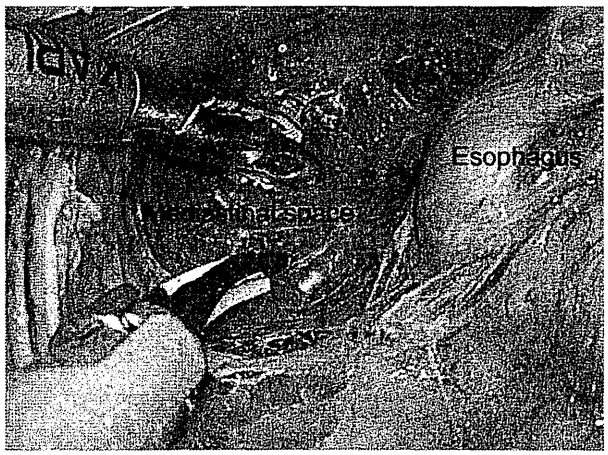

FIGURE 1. Transhiatal dissection of the distal esophagus in the mediastinum.

operating ports were created in the upper abdomen. The left lobe of the liver was retracted using a Penrose drain, as described by Sakaguchi et al.[11] Mobilization of the stomach and perigastric lymphadenectomy were initially performed, depending on the selected operative procedure. The perigastric lymph nodes and lymph nodes around the celiac trunk were removed (around the left gastric artery, common hepatic artery, and proximal side of the splenic artery). The left gastric artery was clipped and divided at the level of its root. Additional splenectomy was not performed. The phrenoesophageal membrane was subsequently divided to expose the abdominal esophagus circumferentially, and the distal esophagus in the mediastinum was then dissected upward from the hiatus and fully mobilized to obtain a sufficient proximal margin from the tumor (Fig. 1). The abdominal esophagus was encircled using cotton tape, which was pulled to stretch the esophageal wall. An anterior incision was sometimes made to the diaphragmatic crus using an ultrasonic coagulating device to widen the esophageal hiatus to improve the view in the mediastinal space. Only the periesophageal lymph nodes were dissected, and the extended mediastinal lymph nodes were not dissected. Intraoperative peroral endoscopy was carried out to determine the transection line of the esophagus. The esophagus was transected using an articulating endoscopic linear stapler (Echelon Flex, Ethicon Endosurgery, Cincinnati, OH) (Fig. 2).

In LTH + LTG, the umbilical port was extended vertically up to 3.5 cm and was protected and retracted using a wound retractor (Alexis Wound Retractor S; Applied Medical, Rancho Santa Margarita, CA). The entire stomach with the distal esophagus was removed through the incision. A 40-cm long Roux limb was then created intracorporeally for subsequent esophagojejunostomy. Jejunojejunal anastomosis (Y anastomosis) was performed using an endoscopic linear stapler (side-to-side).

In LTH + LPG, the left upper port (subcostal) was extended transversely up to 5 cm, through which the proximal stomach with distal esophagus was resected with a linear stapler at the upper third line. A 15-cm-long straight pedicled jejunum was then created for interposition through the mini-laparotomy. Jejunojejunal anastomosis was performed by handsewing under direct vision.

In all the cases, rapid pathologic examination of frozen sections was performed to assess the proximal surgical margins. After reestablishment of pneumoperitoneum, a 25-mm circular stapler anvil head was placed at the stump of the esophagus. In the initial 4 cases, the anvil head was placed by a transabdominal procedure using handsewn purse-string sutures, as described previously.[12] In these cases, a detachable bowel clamp (Endo intestinal clip; Aesculap, Tuttlingen, Germany) was placed at the esophagus proximally as far as possible, avoiding withdrawal of the esophagus into the mediastinum during purse-string suturing. In addition, a monofilament pretied loop was applied to ensure ligation. In the last 6 cases, a transoral delivery system using a pretilted anvil head (Orvil; Covidien, Norwalk, CT) was used, as described for usual LTG or LPG.[13,14] The Roux limb or pedicled jejunum was positioned in retrocolic manner to reduce tension to the anastomosis. Intracorporeal end-to-side esophagojejunostomy was performed using a circular stapler (Fig. 3), the main body of which was introduced through a surgical glove attached to the wound retractor at the mini-laparotomy. The distal stump of the jejunum was closed using a 60-mm endoscopic linear stapler. In LTH + LPG, jejunogastric anastomosis was performed using the 60-mm endoscopic linear stapler at the anterior wall of the gastric remnant in side-to-side manner (Fig. 4). Neither myotomy nor pyloroplasty was performed in the pyloric ring.

RESULTS

To date, 10 patients have successfully undergone LTH resection for AEG type II (2 LTH + LTG and 8 LTH + LPG). Two patients underwent LTH + LTG because they

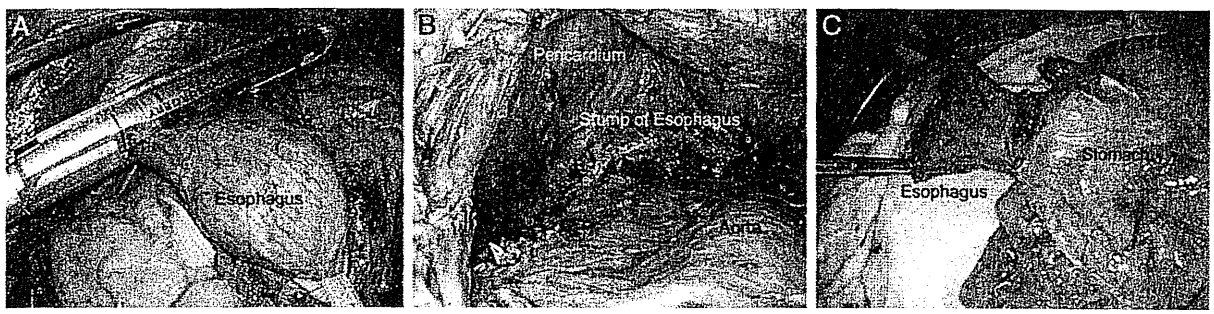

FIGURE 2. A, Transection of the distal esophagus using an articulating linear stapler. B, Mediastinal view after transection of the esophagus. C, Extracted specimen of the proximal stomach with the distal esophagus.

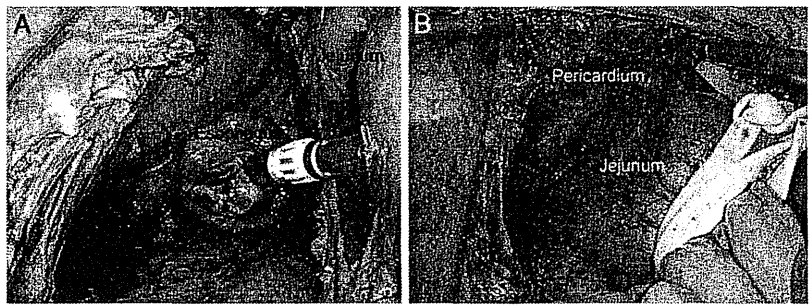

FIGURE 3. A and B, Intracorporeal esophagojejunal anastomosis using a circular stapler.

had synchronous early gastric cancer in the lower stomach. The median age of the patients was 64.1 years (range, 37 to 82 y) and the median body mass index was 23 (range, 18 to 26). The median operation time was 243 minutes (range, 186 to 321 min), the median estimated blood loss was 25.5 g (range, 3 to 108 g), and no transfusions were required. The times of requiring analgesia, in addition to the basal anesthesia, was 1.0 (range, 0 to 2). The median number of retrieved lymph nodes was 22.2 (range, 13 to 35). There were no severe intraoperative complications, and no conversion to open surgery was required in any patient. No postoperative anastomotic leaks or anastomotic stenosis were observed. A diaphragmatic hernia occurred on postoperative day 1 in one LTH + LPG patient, and emergency relaparoscopy was performed the same day. In this case, the omentum, transverse colon, and small intestine migrated to the left thoracic cavity through the enlarged esophageal hiatus. These organs were removed from the abdominal cavity laparoscopically and the enlarged hiatus was then repaired. In another LTH + LPG patient, postoperative gastric stasis developed, with a complaint of gastric fullness; this symptom was improved by conservative treatment, but the patient required a prolonged hospital stay (55 days). The other 9 patients recovered normal activity soon after the surgery, with a median postoperative hospital stay of 13 days (range, 9 to 20 d). They were allowed to take clear liquids on postoperative day 3 and solid food on day 4.

Pathologic findings revealed a median proximal resection margin of 14.5 mm (range, 10 to 23 mm) and a median circumferential margin of 3.6 mm (range, 2.4 to 4.5 mm). The median size of the tumor (maximum diameter) was 25.4 mm (range, 12 to 49 mm) and the median length of esophageal invasion was 18.6 mm (range, 2.5 to 20.0 mm). All the resections were R0, based on the final pathology reports.

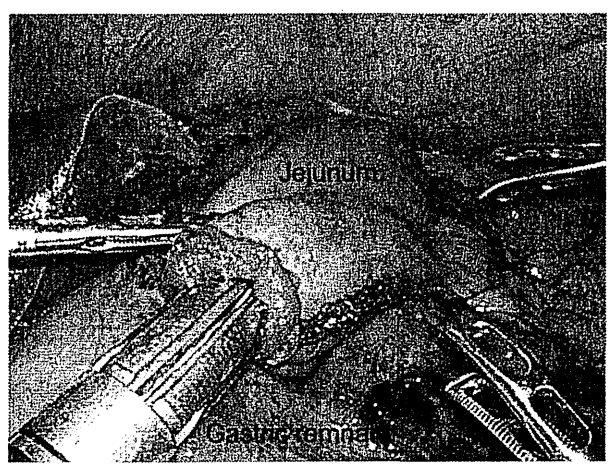

FIGURE 4. Intracorporeal jejunogastric anastomosis using a linear stapler in LTH+LPG. LPG indicates laparoscopic proximal gastrectomy; LTH, laparoscopic transhiatal resection.

DISCUSSION

Some clinical trials have suggested that a transhiatal approach by laparotomy is preferable to thoracotomy for AEG type II. The Japan Clinical Oncology Group (JCOG9502) demonstrated the superiority of the transhiatal approach over left thoracotomy for the treatment of AEG type II and III tumors. This approach was associated with lower morbidity when the length of esophageal invasion was < 3 cm.[8] In this context, we hypothesized that the laparoscopic technique could represent an alternative technique for treating AEG type II; and as for gastric cancer, when the tumor was in early stage and the esophageal invasion was ≤ 3 cm. We started to perform this procedure after the experience of 150 cases of laparoscopic distal gastrectomy and 40 cases of LTG or LPG for gastric cancer.

The concept of LTH esophagectomy was first reported by DePaula et al in 1995[15] and by Swanstrom and Hansen in 1997,[16] and similar operation using the inversion technique have been also reported by other researchers.[17] Montenovo et al[18] reported excellent outcomes of laparoscopic-assisted transhiatal esophagectomy for AEG. However, in all of these reports, reconstruction was performed by cervical anastomosis using the gastric tube. In contrast, reports of LTH resection for AEG with esophagojejunal anastomosis are lacking, probably due to the difficulties associated with anastomotic techniques. Only Patriti et al[19] has reported the preliminary outcomes of robot-assisted LTH resection in 17 patients with cardia cancer, including 3 cases of AEG type II.

From a technical point of view, enhanced laparoscopic visualization of the mediastinal space through the hiatus was thought to be preferable to open surgery, possibly reducing the risk of hemorrhage or complications. Indeed, our experiences suggest that meticulous dissection under a bloodless field could be performed using an ultrasonic coagulating device. In contrast, laparoscopic esophagojejunal anastomosis in the mediastinum is thought to be the most

difficult aspect of this operation. Advances in circular stapling devices have enabled surgeons to safely perform mediastinal anastomosis without using the thoracic approach, but esophagojejunostomy is still thought to be a challenging laparoscopic surgical technique, even in cases of usual LTG or LPG. For usual LTG or LPG, we used handsewn purse-string sutures to place the anvil head at the esophageal end, using detachable intestinal clips.[12] The same method was attempted in the initial 4 cases in this study, but this procedure at the higher esophagus was technically demanding, even when the hiatus was widened. From the fifth case, we therefore switched to transoral placement of the anvil head. This device was originally developed specifically for bariatric surgery, and allows the esophagus to be transected further proximally than before; furthermore, placement of the anvil head in the mediastinum becomes much easier to perform. However, the possible risk of injuring the esophageal wall during transoral delivery represents a potential drawback of this method. In addition, the long-term outcomes of the double-stapling esophagojejunal anastomosis, such as the incidences of leakage or stenosis, have not yet been established. Although sufficient clinical data for this device are lacking, we believe that it presents the most suitable option for higher anastomosis in the mediastinum.

Regarding the postoperative complications of esophagojejunostomy, anastomotic leak with mediastinitis is considered to be the most important and potentially life-threatening one; however, no instances of anastomotic leak occurred in the present series. We believe it is essential to secure the anastomoses to allow sufficient visibility, with adequate widening of the diaphragmatic crus. One diaphragmatic herniation (in the sixth case) occurred as a result of this enlargement. This complication has been also reported in esophagectomy with gastric tube replacement.[20] The enlarged diaphragmatic crus was meticulously repaired by suturing to prevent this complication in subsequent cases in the present series.

The oncological suitability of the laparoscopic procedure for treating AEG type II also needs to be evaluated. A safe proximal margin would minimize the anastomotic recurrence rate.[21] In our series, the adequate transection point was confirmed by intraoperative endoscopy, and the pathologic findings indicated that safe surgical margins as for T1 tumors were obtained in all patients. Several studies have also emphasized the importance of the circumferential margin as a prognostic factor in the surgical treatment of AEG,[22] and the distance of the circumferential margin was also satisfactory in our series. The median number of regional lymph nodes retrieved was 22.9, which was also satisfactory. The lymphatic spread of AEG was clearly demonstrated in a large-scale study by Siewert et al,[3] who reported lymphatic involvement of type II tumors mainly in the left (67%) and right (63%) paracardial regions, lesser curvature (66%), and toward the branch of the celiac trunk (25%). The reported occurrence of lower mediastinal lymph node metastasis from type II is 12%, but the incidence in T1 tumors is reported to be very low.[23] The range of lymph node metastases in the present series was similar to those in usual LTG or LPG for gastric cancer.

Regarding the quality of life, the cosmetic results were excellent after our procedure. All the patients recovered quickly and postoperative analgesia was minimized. No pulmonary-associated complications were recorded, probably due to the minimal damage to the body wall. In addition, gastric reservoir function was preserved in 8 patients who underwent LPG. Such function-preserving surgery through minimal access may further contribute to the patient quality of life.

Our preliminary experiences suggest that advances in instrumentation mean that LTH resection of localized AEG type II is technically feasible and can be performed safely after adequate experience of performing LTG or LPG for gastric cancer. However, it remains a complex, advanced laparoscopic procedure, with esophagojejunal anastomosis in the mediastinum being especially technically demanding. This procedure should presently only be performed by experienced laparoscopic surgeons. More cases need to be examined and future, prospective clinical trials may be needed to assess the benefits of these surgical techniques.

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Pancreatology 12 (2012) 428–433

Contents lists available at SciVerse ScienceDirect

Pancreatology

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Original article

Treatment outcome for systemic chemotherapy for recurrent pancreatic cancer after postoperative adjuvant chemotherapy

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ARTICLE INFO

Article history:
Received 22 May 2012
Received in revised form
16 July 2012
Accepted 17 July 2012

Keywords:
Adjuvant chemotherapy
Chemotherapy
Gemcitabine
Recurrent pancreatic cancer
S-1

ABSTRACT

Objectives: A global consensus on how to treat recurrent pancreatic cancer after adjuvant chemotherapy with gemcitabine (ADJ-GEM) does not exist.
Methods: We retrospectively reviewed the clinical data of 41 patients with recurrences who were subsequently treated with chemotherapy.
Results: The patients were divided into two groups according to the time until recurrence after the completion of ADJ-GEM (ADJ-Rec): patients with an ADJ-Rec < 6 months ($n = 25$) and those with an ADJ-Rec ≥ 6 months ($n = 16$). The disease control rate, the progression-free survival after treatment for recurrence and the overall survival after recurrence for these two groups were 68 and 94% ($P = 0.066$), 5.5 and 8.2 months ($P = 0.186$), and 13.7 and 19.8 months ($P = 0.009$), respectively. Furthermore, we divided the patients with an ADJ-Rec < 6 months into two groups: patients treated with gemcitabine ($n = 6$) and those treated with alternative regimens including fluoropyrimidine-containing regimens ($n = 19$) for recurrent disease. Patients treated with the alternative regimens had a better outcome than those treated with gemcitabine.
Conclusions: Fluoropyrimidine-containing regimens may be a reasonable strategy for recurrent disease after ADJ-GEM and an ADJ-Rec < 6 months.

1. Introduction

Pancreatic cancer patients have an extremely poor prognosis. Although surgical resection is the only curative treatment, only 15%–20% of patients are candidates for resection. Even if a curative resection is performed, the 5-year-survival rate is only 10%–25%, and the median survival period is 11–20 months [1,2].

Various adjuvant chemotherapy or chemoradiotherapy regimens after surgical resection have been evaluated [2–6]. Recently, The Charite' Onkologie (CONKO)-001 trial was designed to determine the benefits of gemcitabine for patients with resected pancreatic cancer. Adjuvant chemotherapy with gemcitabine (ADJ-GEM) significantly improved the disease-free survival period, compared with surgery alone, in patients with resected pancreatic cancer. Although no significant difference in overall survival was seen at the time of publication, analysis after a longer follow-up period demonstrated a survival advantage for gemcitabine over observation-only (median progression-free survival, 22.8 months for ADJ-GEM vs. 20.2 months for observation-only; $P = 0.005$). At approximately the same time as the CONKO-001 trial, the Japanese Study Group of Adjuvant Therapy for Pancreatic Cancer (JSAP) conducted a randomized clinical trial evaluating adjuvant gemcitabine. Although no significant difference in overall survival was seen, the patients in the gemcitabine arm demonstrated a significantly longer disease-free survival period than the patients in the observation-only arm. These results were similar to those of the CONKO-001 trial and supported the concept that adjuvant chemotherapy using gemcitabine was effective in an Asian

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1424-3903/$ – see front matter
http://dx.doi.org/10.1016/j.pan.2012.07.016

population [2,5]. Therefore, adjuvant therapy using gemcitabine for resected pancreatic cancer is now firmly established as a therapy that offers a modest but real improvement in overall survival [5,7].

In approximately 50% of patients, recurrent disease was reportedly seen within a year, even after receiving ADJ-GEM [5], and no global consensus exists regarding treatment strategies for recurrent disease after ADJ-GEM. If the length of time from the completion of adjuvant therapy until the detection of recurrence is less than 6 months, the NCCN guidelines recommend alternative chemotherapy using a fluoropyrimidine-based chemotherapy regimen. When this period is 6 months or greater, they recommend an alternative regimen or the same regimen as the previous therapy [8]. However, these recommendations have not been substantiated by actual clinical data.

In Japan, the oral fluoropyrimidine derivative S-1 is often used as an alternative regimen for gemcitabine-refractory cases. S-1 showed a non-inferiority to gemcitabine in terms of overall survival in a phase III trial and is considered an alternative to gemcitabine for chemonaïve patients with advanced pancreatic cancer [9]. Additionally, in gemcitabine-refractory metastatic cases, a recent phase II study of S-1 yielded results that demonstrated preferable activity, including a response rate of 9.5%–15% and a median overall survival time of 4.5–6.3 months [10,11]. Therefore, S-1 is widely used for the treatment of advanced pancreatic cancer in first-line and second-line settings in Japan.

We studied the current status of treatments for recurrent pancreatic cancer after curative resection followed by ADJ-GEM. The objective of this study was to examine the adequacy of the

Table 1
Patient characteristics at resection ($n = 41$).

		n (%)			
	Variables	All patients $n = 41$	ADJ-Rec < 6 months $n = 25$	ADJ-Rec ≥ 6 months $n = 16$	*P* value
Age (years)	Median (range)	65 (38–78)	64 (38–78)	65 (50–77)	0.96
Gender	Male	27 (66)	16 (64)	11 (69)	1.00
	Female	14 (34)	9 (36)	5 (31)	
PS[a] at recurrence	0	30 (73)	20 (80)	10 (63)	0.34
	1	5 (12)	3 (12)	2 (12)	
	Unknown	6 (15)	2 (8)	4 (25)	
Primary site	Head	26 (63)	17 (68)	9 (56)	0.51
	Body or -tail	15 (37)	8 (32)	7 (44)	
Type of Resection	PD[b]	26 (64)	17 (68)	9 (56)	0.66
	DP[c]	12 (29)	6 (24)	6 (38)	
	TP[d]	3 (7)	2 (8)	1 (6)	
Resection status	R0	36 (88)	22 (88)	14 (88)	1.00
	R1	5 (12)	3 (12)	2 (12)	
Histology	Adenocarcinoma	39 (95)	23 (92)	16 (100)	0.51
	Adenosquamous carcinoma	2 (5)	2 (8)	0 (0)	
Stage[e] at resection	IIA	5 (12)	0 (0)	5 (31)	0.006
	IIB	36 (88)	25 (100)	11 (69)	
CEA[f] (ng/mL)	Median (range)	2.7 (0.7–51.8)	2.7 (0.7–21.0)	2.4 (1.2–51.8)	0.98
CA19-9[g] (U/mL)	Median (range)	202 (0.5–6450)	212 (0.5–6450)	138 (17–3203)	0.56
Histological grade	Well	5 (12)	3 (12)	2 (12.5)	0.83
	Moderately	28 (71)	17 (68)	12 (75)	
	Poorly	7 (17)	5 (20)	2 (12.5)	
Lymph node ratio[h]	0	5 (12)	0 (0)	5 (31)	0.008
	0.1–0.199	23 (56)	14 (56)	9 (57)	
	0.2–0.299	8 (20)	7 (28)	1 (6)	
	0.3–	4 (10)	4 (16)	0 (0)	
	Unknown	1 (2)	0 (0)	1 (6)	
Recurrent pattern[i]	Locoregional	21 (51)	10 (40)	11 (69)	0.15
	Liver	18 (44)	14 (56)	4 (25)	
	Peritoneum	4 (10)	4 (16)	0 (0)	
	Lungs	11 (27)	7 (28)	4 (25)	
	Bones	1 (2)	1 (4)	0 (0)	
Cycles of ADJ-GEM	Median (range)	6 (3–9)	6 (3–6)	6 (3–9)	0.88
ADJ-Rec[j] (months)	Median (range)	3.7(0.1–36.1)	1.3 (0.1–4.9)	11.5 (6.3–36.1)	
Chemotherapy[k]	GEM	21 (51)	6 (24)	15 (94)	0.00
	Alternatives[l]	20 (49)	19 (76)	1 (6)	
	(S1)	17 (41)	17 (68)	1 (6)	
	(GEM + S1)	1 (2)	0 (0)	0 (0)	
	(S1 + Radiation)	1 (2)	1 (4)	0 (0)	
	(S1 + oxaliplatin)	1 (2)	1 (4)	0 (0)	

[a] PS, performance status.
[b] PD, pancreaticoduodenectomy.
[c] DP, distal pancreatectomy.
[d] TP, total pancreatectomy.
[e] Stage, UICC 7th.
[f] CEA, carcinoembryonic antigen at resection.
[g] CA-19-9, carbohydrate antigen 19-9 at resection.
[h] Lymph node ratio, number of metastatic lymph nodes divided by number of examined nodes.
[i] Recurrent pattern, numbers of locoregional, extra-pancreatic, and combined recurrences were 11, 20, and 10 patients.
[j] ADJ-Rec, period between the last date of ADJ-GEM and recurrence.
[k] Chemotherapy, chemotherapy for recurrent disease after adjuvant chemotherapy.
[l] Alternatives, all alternative regimens consisted of fluoropyrimidine-containing regimens.

NCCN guidelines for recurrent pancreatic cancer after adjuvant chemotherapy, which recommend that the treatment options should be determined by the period between the last date of ADJ-GEM and recurrence (ADJ-Rec), with a threshold of 6 months.

2. Patients and methods

2.1. Patients

A retrospective review was conducted for 113 pancreatic cancer patients who underwent curative resection followed by ADJ-GEM at the National Cancer Center Hospital (NCCH) and NCCH East in Japan between April 2002 and October 2010. Forty-two patients with no recurrence after ADJ-GEM, 10 patients with withdrawal from ADJ-GEM within 2 cycles, 6 patients with recurrence during ADJ-GEM, and 14 patients who changed hospitals after recurrence were excluded. We finally retrieved the clinical data of 41 patients with recurrences who were subsequently treated with chemotherapy at our hospitals.

2.2. Treatment

After resection, we started ADJ-GEM within 10 weeks. An initial gemcitabine dose of 1000 mg/m^2 was administrated intravenously for 30 min on days 1, 8 and 15 every 4 weeks for 3 to 6 cycles, in principle. A computed tomography examination was performed every 3–6 months. Once evidence of recurrence was revealed, treatment for recurrent disease was initiated.

2.3. Data collection and evaluation of tumor response

The following data were collected from the medical records: patient characteristics at resection, the resection status, the ADJ-Rec, the treatment regimen, and the outcome of treatment after the recurrence. We also compared the treatment outcomes according to the length of the ADJ-Rec and the treatment regimens. Tumor responses were evaluated according to the RECIST criteria, Ver.1.1. We evaluated the best overall response and the disease control rate (DCR). The DCR was defined as the rate of complete response + partial response + stable disease. When the disease status was stably maintained for more than 8 weeks, the patient was considered to have stable disease.

2.4. Statistical analysis

The Fisher exact test was used to assess the hypothesis of independence between categorical variables. For quantitative data such as age and the carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) levels, we used the Mann–Whiney test. ADJ-Rec was defined as the period between the last date of the administration of ADJ-GEM and the date on which local or distant recurrence was noted. The date of recurrence was defined as the date of documentation of recurrent disease using diagnostic imaging techniques. Progression-free survival (PFS) was defined as the period between the start of treatment for recurrent disease and the date of progression, the last follow-up visit, or death from any cause. Overall survival after recurrence (r-OS) was defined as the period between the start of treatment for recurrent disease and death from any cause or the last follow-up. Patients who were lost to follow-up were treated as censored cases. Survival curves were estimated using the Kaplan–Meier method, and the significances were evaluated using a log-rank test. All the analyses were performed using Stata/SE, Version 11.1 (StataCorp, USA).

3. Results

3.1. Patient characteristics

The characteristics at resection of the 41 eligible patients are listed in Table 1. R0 resection (complete resection with no microscopic residual tumor) was performed in 36 patients (88%). Concerning the pathological stage, 5 (12%) of the patients had stage IIA disease and 36 (88%) had stage IIB. The sites of recurrence were locoregional (21 patients), the liver (18 patients), and the lung (11 patients). Patients with an ADJ-Rec $\geq$ 6 months (16 patients) had a significantly better status than patients with an ADJ-Rec < 6 months (25 patients) with regard to disease stage ($P = 0.006$) and the lymph node ratio (the number of metastatic lymph nodes divided by the number of examined nodes) ($P = 0.0075$). As for the treatments for recurrent disease, 21 patients were treated with gemcitabine monotherapy and 20 patients were treated with alternative regimens. All the alternative regimens were fluoropyrimidine-containing regimens (17 patients received S-1 and 1 patient each received GEM + S-1, S-1 + radiation, and S-1 + oxaliplatin). The treatment strategy after recurrence depended on each oncologist's plan, without a unified policy. Among the 25 patients with an ADJ-Rec < 6 months, 6 were treated with gemcitabine monotherapy and 19 were treated with alternative regimens. Among the 16 patients with an ADJ-Rec $\geq$ 6 months, 15 were treated with gemcitabine monotherapy and 1 was treated with an alternative regimen.

3.2. Treatment efficacy and survival analysis of treatments for recurrence

Overall, 2 of the 41 patients responded to the treatments for recurrent disease (4.9%; 2 partial responses; 95% confidence interval (95% CI), 0.60%–16.53%). The DCR was 78% (32 of the 41 patients; 95% CI, 62.39%–89.44%). The median PFS and median r-OS were 5.5 months (95% CI, 3.7–8.1 months) and 18.3 months (95% CI, 13–19.8 months), respectively (Fig. 1).

We divided the patients into two groups according to the length of the ADJ-Rec: patients with an ADJ-Rec < 6 months ($n = 25$), and patients with an ADJ-Rec $\geq$ 6 months ($n = 16$). The DCRs were 68% and 94% ($P = 0.066$), and the median PFS periods were 5.5 and 8.2 months ($P = 0.186$; Fig. 2A), respectively. The median r-OS of the patients with an ADJ-Rec < 6 months was significantly shorter than

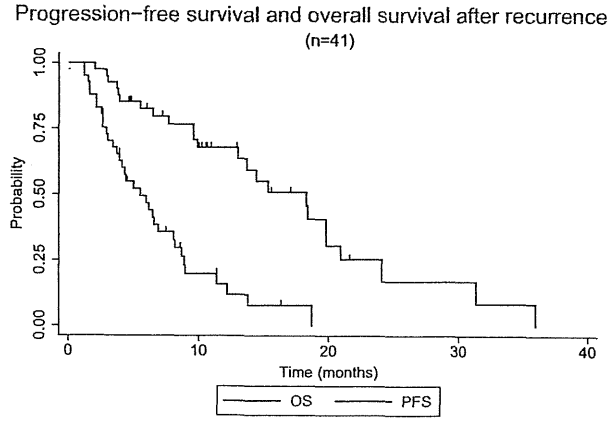

Fig. 1. Progression-free survival (PFS) and overall survival after recurrence (r-OS) in all patients ($n = 41$). The median PFS and r-OS were 5.5 and 18.3 months, respectively.

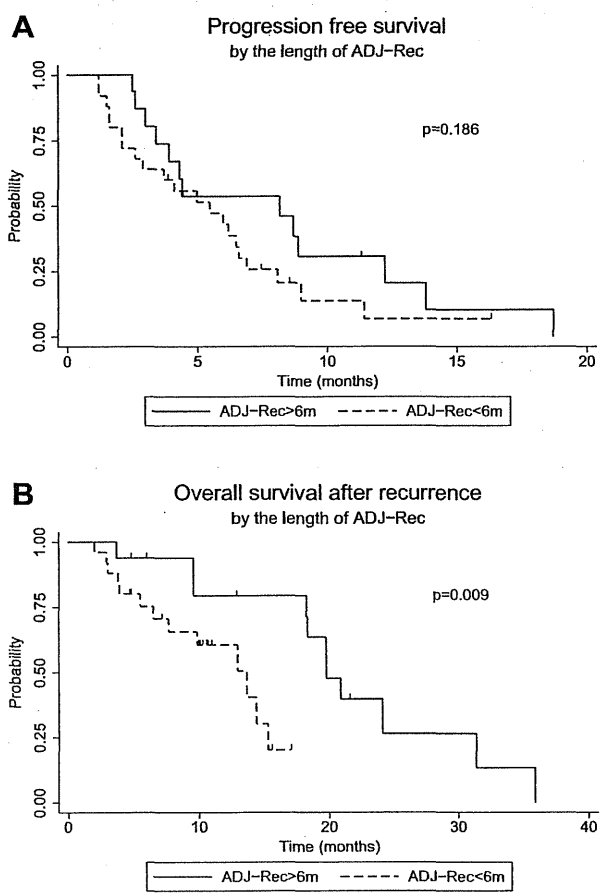

Fig. 2. Progression-free survival (PFS) and overall survival after recurrence (r-OS) according to the length of the ADJ-Rec: patients with an ADJ-Rec < 6 months ($n = 25$), and patients with an ADJ-Rec ≥ 6 months ($n = 16$). (A) The median PFS for each group was 5.5 and 8.2 months ($P = 0.186$), respectively. (B) The median r-OS was 13.7 and 19.8 months ($P = 0.009$), respectively.

that of the patients with an ADJ-Rec ≥ 6 months (13.7 and 19.8 months, $P = 0.009$; Fig. 2B).

Additionally, we divided the patients with an ADJ-Rec < 6 months into two groups according to the treatment regimens for recurrent disease: patients treated with gemcitabine ($n = 6$) and patients treated with alternative regimens ($n = 19$). The outcomes are shown in Table 2 and Fig. 3. For the patients treated with gemcitabine and those treated with alternative regimens, the DCR, median PFS and median r-OS were 67% and 68% ($P = 0.651$), 2.9 and 6.5 months ($P = 0.065$; Fig. 3A), and 7.7 and 13.0 months ($P = 0.242$; Fig. 3B), respectively.

4. Discussion

In this study, at first we examined the current status of the treatment strategy for pancreatic cancer patients with recurrence after adjuvant chemotherapy. Most patients with ADJ-Rec ≥ 6 months were placed on gemcitabine. Even for patients with an ADJ-Rec < 6 months, gemcitabine was resumed in 24% of these patients. Generally, patients who relapse within a short period after receiving adjuvant chemotherapy should be considered as being resistant to those drugs. The NCCN guidelines also recommend that the options for recurrent disease after adjuvant therapy should be assessed according to the ADJ-Rec. However, these guidelines are only the recommendation of the panel, and these strategies have not yet been substantiated by actual clinical data. In the case of ovarian cancer, a consensus based on actual clinical data exists with regard to the treatment strategy for relapsed disease. Patients who have relapsed within an interval of less than 6 months since the previous paclitaxel-plus-platinum chemotherapy should be considered as platinum resistant [12,13]. However, the chemosensitivity and the key drugs are quite different between pancreatic cancer and ovarian cancer. Therefore, actual clinical data for pancreatic cancer is needed.

The outcome of patients with a short ADJ-Rec was worse than that of the patients with a long ADJ-Rec. This finding suggests that patients with a long ADJ-Rec may owe their period of prolonged sensitivity to the adjuvant gemcitabine treatment, slow tumor growth, and a smaller quantity of residual tumor. Concerning advanced pancreatic cancer, similar findings have been reported in a previous study, which indicated that the progression-free survival period after first-line chemotherapy was an independent prognostic factor [14]. Additionally, patients with pathological stage IIA or a lymph node ratio of 0 had a long ADJ-Rec in the present study, possibly influencing the outcome. However, our results should be interpreted with caution because biases introduced by the different selection of treatment regimens between the two groups may exist.

Among the patients with an ADJ-Rec ≥ 6 months, we were unable to compare the treatment outcome according to regimens, since most of them (15 out of 16) received gemcitabine monotherapy and seldom received alternative options such a fluoropyrimidine-based regimens. In the present study, the patients treated with gemcitabine had a better DCR, PFS and r-OS than the metastatic or recurrent pancreatic cancer patients treated with gemcitabine in past studies [15,16]. Even after considering the possibility that an ADJ-Rec ≥ 6 months may be a good prognostic factor, these preferable outcomes suggest the appropriateness of a re-challenge with gemcitabine.

Among the patients with an ADJ-Rec < 6 months, patients receiving alternative regimens tended to have a better DCR, PFS,

Table 2
Outcomes of patients according to ADJ-Rec and treatment regimens.

	<6 months				≥6 months			
ADJ-Rec	All	GEM	Alternative	*P* value	All	GEM	Alternative	*P* value
n	25	6	19		16	15	1	
DCR (%)	68	67	68	1.00	94	93	(100)	1.00
95% CI	62.4–89.4	22.3–95.7	43.5–87.4		69.8–99.8	68.1–99.8	2.5–100	
Median PFS (m)	5.5	2.9	6.5	0.06	8.2	8.2	(12.2)	0.69
95% CI	2.6–6.6	1.5–	2.1–8.1		3.4–12.2	3.0–13.8		
Median r-OS(m)	13.7	7.7	13.0	0.24	19.8	20.9	(19.8)	0.67
95% CI	6.5–15.3	2.9–	6.5–		9.6–31.4	9.6–31.4		

ADJ-Rec, period between the last date of ADJ-GEM and recurrence; DCR, disease control rate; PFS, progression-free survival time; r-OS, survival time from recurrence; Alternative*, including S-1, GEM + S-1, S-1 + radiation, and S-1 + oxaliplatin.

and r-OS than those receiving gemcitabine monotherapy. Although the optimal ADJ-Rec threshold was not clarified, the present results support the recommendations of the NCCN guidelines, which recommend alternative regimens for patients with an ADJ-Rec < 6 months after previous treatment with gemcitabine. These findings suggest that a certain proportion of patients with a short ADJ-Rec may already have a gemcitabine-refractory status at the time of ADJ-GEM.

This study had some limitations. This study was a retrospective analysis with an insufficient sample size, and the treatment strategy after recurrence depended on each oncologist's plan, with no unified policy. Another limitation concerns the alternative treatment options after recurrence. The NCCN guidelines recommend alternative regimens as second-line therapies for metastatic disease. The recommended regimens consist of fluoropyrimidine-based therapies, such as 5-FU/leucovorin (LV)/oxaliplatin (Oxal) [17] or capecitabine/Oxal [18]. The CONKO-003 study revealed the survival advantage of 5-FU + LV + Oxal for gemcitabine-refractory pancreatic cancer. In Japan, these drugs have not yet been approved under the Japanese medical insurance system for the treatment of pancreatic cancer. S-1 monotherapy was mainly used as the alternative option in our study. Although S-1 demonstrated a non-inferiority to gemcitabine as a first-line treatment [8,9] and had a marginal activity as a second-line regimen for gemcitabine-refractory pancreatic cancer [10,11], it has not been accepted as a global standard therapy for gemcitabine-refractory pancreatic cancer.

In conclusion, patients with an ADJ-Rec ≥ 6 months had a relatively favorable outcome when treated with a gemcitabine rechallenge. Among the patients with an ADJ-Rec < 6 months, those patients receiving alternative regimens tended to have a better DCR, PFS, and r-OS, compared with those receiving gemcitabine. As a result, our results did not deny the appropriateness of strategies outline in the NCCN guidelines. A well-designed prospective study with a sufficient sample size is needed to identify the optimal regimen for the treatment of recurrent pancreatic cancer after postoperative adjuvant chemotherapy.

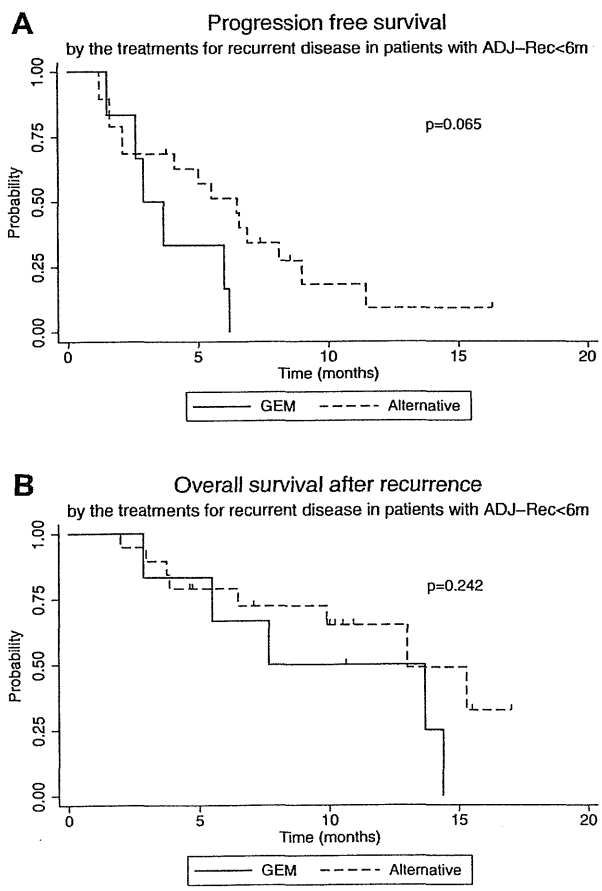

Fig. 3. Progression-free survival (PFS) and overall survival after recurrence (r-OS) according to treatments for recurrent disease in patients with an ADJ-Rec < 6 months: patients treated with gemcitabine ($n = 6$), and patients treated with alternative regimens ($n = 19$). (A) The median PFS for each group was 2.9 and 6.5 months ($P = 0.065$), respectively. (B) The median r-OS was 7.7 and 13.0 months ($P = 0.242$), respectively.

Grant support

None declared.

Conflict of interest

Takuji Okusaka had research findings and honoraria to disclose from Taiho pharmaceutical co. and Eli Lilly Japan.

Hideki Ueno had honoraria to disclose from Taiho pharmaceutical co. and Eli Lilly Japan, and had a consultation or advisory relationship to disclose from Taiho phatmaceutical co.

Tomoo Kosuge had honoraria to disclose from Taiho pharmaceutical co. and Eli Lilly Japan.

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Cancer Science
The official journal of the Japanese Cancer Association JCA

Impact of tumor-associated macrophages on invasive ductal carcinoma of the pancreas head

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(Received February 25, 2012/Revised July 4, 2012/Accepted July 18, 2012/Accepted manuscript online August 30, 2012/Article first published online October 4, 2012)

Tumor-associated macrophages (TAMs) are candidate histological factors in invasive ductal carcinoma (IDC) of the pancreas. Tumor-associated macrophages can be affected by cancer-related inflammation and pancreatitis and interact with important invasive behavior in a recurrent manner in pancreatic IDC. These features may help elucidate the aggressiveness of pancreatic IDC. The aim of this study was to characterize TAMs in pancreatic IDC in comparison with chronic pancreatitis (CP) and to reveal TAM-related factors and the clinical impact of TAMs. CD68 (a pan-macrophage marker) and CD204 (an M2 macrophage marker) immunohistochemistry was carried out in pancreas head specimens from 107 IDC cases and 11 CP cases. Immunopositive cell areas were calculated at the periphery and center of the tumor. The distributions of macrophages in IDC and CP and the relationship between TAMs and histological tumor factors, survival, and recurrence were evaluated. Macrophages were more frequently observed in the lesion periphery than the center in IDC and CP. The density of macrophages was elevated in IDC compared to CP. Dense M2 macrophages at the tumor periphery were frequently seen in large tumors and showed an independent impact on overall survival and disease-free time. Early recurrence in the liver or the local manipulated area was associated with high accumulation of peripheral M2 macrophages. More M2 macrophages were seen in IDC than in CP in both the periphery and the center. High numbers of peripheral M2 macrophages were associated with large tumor size, early recurrence in the liver, local recurrence, and shortened survival time in patients with pancreatic IDC. (*Cancer Sci* 2012; 103: 2012–2020)

The prognosis of patients undergoing resection for pancreatic invasive ductal carcinoma (IDC) remains poor.[1–5] Histological studies have been carried out to elucidate the aggressiveness of pancreatic IDC and have revealed prognostic factors including tumor size, lymph node involvement, nerve plexus invasion, positive resected margin, and low tumor grade.[1–8] Tumor-associated macrophages (TAMs) have recently been reported as a candidate factor in poor prognosis.[9]

Macrophages are the most abundant cancer stromal cells involved in the host immune system,[10] and TAMs have been found to play important roles in tumorigenesis, angiogenesis, matrix remodeling, and metastasis.[11–13] Tumor-associated macrophages have a prognostic impact in prostate, breast, and lung cancers, as well as pancreatic IDC.[9,14–16] The heterogeneity of macrophages has been discussed with regard to their different responses to various microenvironmental stimuli. Macrophages are classically activated towards the M1 phenotype by lipopolysaccharide and interferon-γ. M1 macrophages are characterized by high expression of pro-inflammatory cytokines, such as interleukin (IL)-1, IL-6, IL-12, and tumor necrosis factor. Alternatively, macrophages are activated towards the M2 phenotype by IL-4, IL-13, and IL-10. M2 macrophages are characterized by high expression of IL-4 and IL-10 and low expression of IL-12.[12] Recent studies have revealed high CD204 expression in M2 macrophages and have shown that TAMs are polarized to the M2 phenotype.[12,17,18]

The distribution of TAMs was recently evaluated as a prognostic index in various cancers. A high number of TAMs in the peripheral area of the tumor is correlated with poor prognosis in gastric cancer,[19] hepatocellular carcinoma,[20] and non-small-cell lung cancer,[21] although an increased number of TAMs in the invasive front of colon cancer is associated with favorable prognosis.[22] Increased numbers of TAMs in many cancers are linked to reduced patient survival. In pancreatic IDC, high accumulation of TAMs in the periphery of the tumor is correlated with extrapancreatic invasion, lymph vessel invasion, lymph node involvement, and shortened survival time.[9] Tumor-associated macrophages may be a key to elucidating the aggressiveness of pancreatic IDC. Detailed clinicopathological studies should be carried out to estimate the role of TAMs. First, the distribution of macrophages should be compared between mass-forming chronic pancreatitis (CP) and pancreatic IDC. Macrophages accumulate at the inflammatory site and play crucial roles in the diverse phase.[23,24] Pancreatitis is prevalent in pancreatic IDC and CP due to obstruction of the main pancreatic duct.[25] Tumor-associated macrophages in pancreatic IDC can be affected by both pancreatitis and inflammatory mediators from tumor cells; macrophages in CP are affected by pancreatitis only. The comparison of macrophages between pancreatic IDC and CP may provide evidence that tumor cells mainly lead to TAM accumulation in pancreatic IDC. Second, TAM-related tumor factors should be examined in detail. Tumor-associated macrophages are attracted to and retained in avascular and necrotic areas where they are exposed to tumor hypoxia.[26,27] Our previous clinicopathological study showed that tumor necrosis is frequent in large tumors.[7] Tumor size may be associated with TAM accumulation. Identification of the precise TAM-related tumor factors is useful for estimating microenvironmental interactions between TAMs and pancreatic IDC. Third, the impact of TAMs on tumor relapse should be evaluated. The prognostic value of TAMs may indicate that TAMs are predictive markers of recurrence. The impact of TAMs on recurrence will reinforce the clinical significance of TAMs. Finally, multivariate analysis should be carried out to confirm the impact of TAMs on prognosis. The prognostic value of TAMs has only been tested with univariate analysis. Establishment of the prognostic importance needs to show independence among various tumor factors with multivariate analysis.

The aim of this study was to characterize TAMs in pancreatic IDC in comparison with CP and to reveal TAM-related

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doi: 10.1111/j.1349-7006.2012.02411.x

Table 1. Characteristics of patients who underwent pancreaticoduodenectomy with curative intent for a pancreatic head tumor

Parameter	Invasive ductal carcinoma	Chronic pancreatitis
Number	107	11
Age (years), median (range)	64.0 (37–82)	52.0 (38–72)
Gender (male/female)	64/44	10/1
CEA (ng/mL), median (range)	3.5 (0.8–60.3)	3 (0.9–15.7)
CA19-9 (U/mL) (median, range)	109.0 (1.0–21400.0)	14.0 (5.0–245.8)
Combined resection (portal vein/inferior vena cava/colon/liver)	51/2/2/2	0
Intraoperative radiotherapy	30	0
Adjuvant chemotherapy	10 (GEM:8, S-1:2)	0
Stage (UICC 6th) (IA/IB/IIA/IIB/III/IV)	0/0/19/79/1/8	

S-1, an oral anti-cancer drug that combines tegafur, a prodrug of fluorouracil, with 5-chloro-2,4-dihydropyrimidine and potassium oxonate in a molar ratio of 1.0:0.4:1.0 (Taiho Pharmaceutical, Tokyo, Japan). CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; GEM, gemcitabine.

factors and the clinical impact of TAMs on tumor relapse and prognosis.

Materials and Methods

Patients. Between September 1992 and December 2007, 116 patients with a pathological diagnosis of pancreatic IDC who underwent a pancreaticoduodenectomy with curative intent at our institution were investigated, because pancreatitis due to obstruction of the main pancreatic duct is evident in the pancreatic head lesions of IDC and CP cases. Three in-hospital deaths, two patients with incomplete follow-up data, two patients who died of non-cancerous causes within 5 years of the pancreaticoduodenectomy (one due to liver cirrhosis and one due to brain infarction), and two patients whose surgical specimens were of poor quality were excluded from the study. The remaining 107 patients were investigated. For the CP cases in this study, 11 patients who underwent pancreaticoduodenectomy during the same period and were pathologically diagnosed with CP were assessed. Chronic pancreatitis was diagnosed according to The Revised Japanese Clinical Diagnostic Criteria for Chronic Pancreatitis.[28] All CP cases showed fibrosis that was distributed primarily in the interlobular spaces, showing a nodular pattern of lobules called cirrhosis due to the disruption of dense interlobular fibrosis or the loss of exocrine parenchyma with irregular fibrosis. All patients signed an institutional review board-approved informed consent form. The median age of the IDC patients was 64.0 years (range, 37–82 years), and 44 were women (41.1%). The median age of the CP patients was 52.0 years (range, 38–72 years), and 1 (9.1%) was a woman (Table 1). None of the 107 IDC patients received neoadjuvant chemotherapy or radiotherapy; 30 received intraoperative radiotherapy,[29] and 10 received adjuvant chemotherapy. Extended lymphadenectomy including regional and peripancreatic lymph node dissection was carried out with pancreaticoduodenectomy, according to the Japanese Classification of Pancreatic Cancer.[30] Combined resection of the portal vein, inferior vena cava, colon, and para-aortic lymph node was carried out for macroscopically curative resection.

To assess initial recurrence of the tumor, follow-up contrast computed tomography was done every 3 months after surgery or earlier if clinically indicated by examination, symptoms, or a rise in tumor markers, such as serum carcinoembryonic antigen and serum carbohydrate antigen 19-9, which were checked every month. If necessary, further examination such as cytology was carried out to diagnose peritoneal dissemination.

Evaluation of clinicopathological features. Clinical characteristics and pathological examination results were retrieved from the clinical records. Lymphatic (ly), venous (v), and intrapancreatic nerve invasion (ne) were classified into four groups according to the definition of the Japan Pancreas Society and were based on the most extensively involved area observed

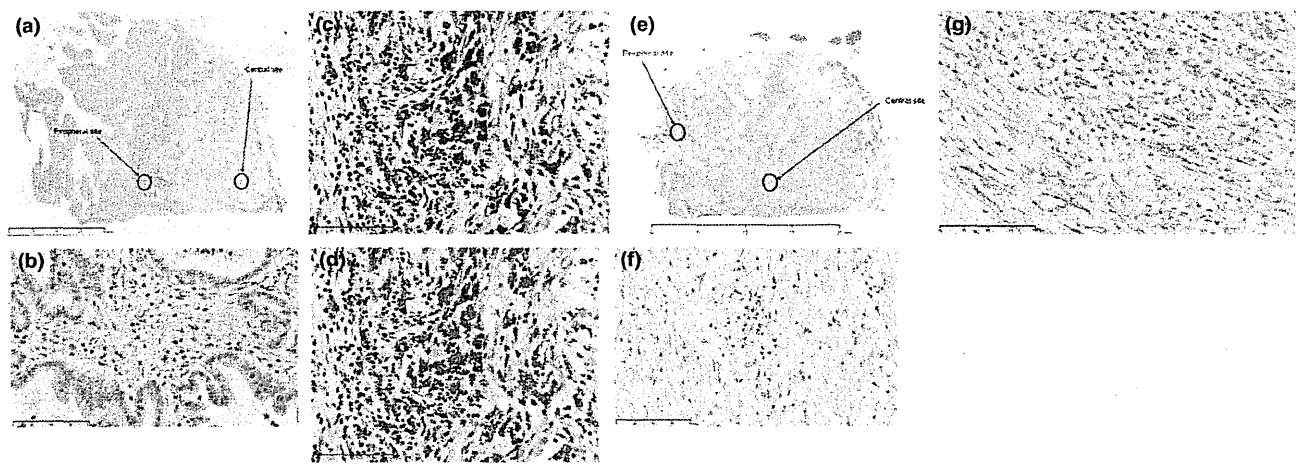

Fig. 1. Objective measurement of the area ratio of immunopositive cells. (a) Using the section showing the maximum diameter of the invasive ductal carcinoma tumor that was stained with anti-CD204, hot spots in the center and the periphery of the tumor were observed at a magnification of ×40. Center (b) and periphery (c) of invasive ductal carcinoma of the pancreas (magnification, ×400). We measured the area of immunopositive cell bodies at this magnification using the Automeasure function of Axio Vision 4.7.1. Axio Vision software visualized the CD204-positive area as red-colored areas (d) and objectively calculated the positive area ratio (summed area of immunopositive cells/measured area). CD204 expression in chronic pancreatitis (CP) tumors was measured using the section with the maximum diameter of the CP tumor (e–g). In the entire image of the CD204-stained CP section (E; magnification, ×40), hot spots of CD204 expression at the central and the peripheral sites of the CP (f, g; magnification ×400) were selected. The positive area ratio of CD204 in the selected image was objectively calculated with Axio Vision software.

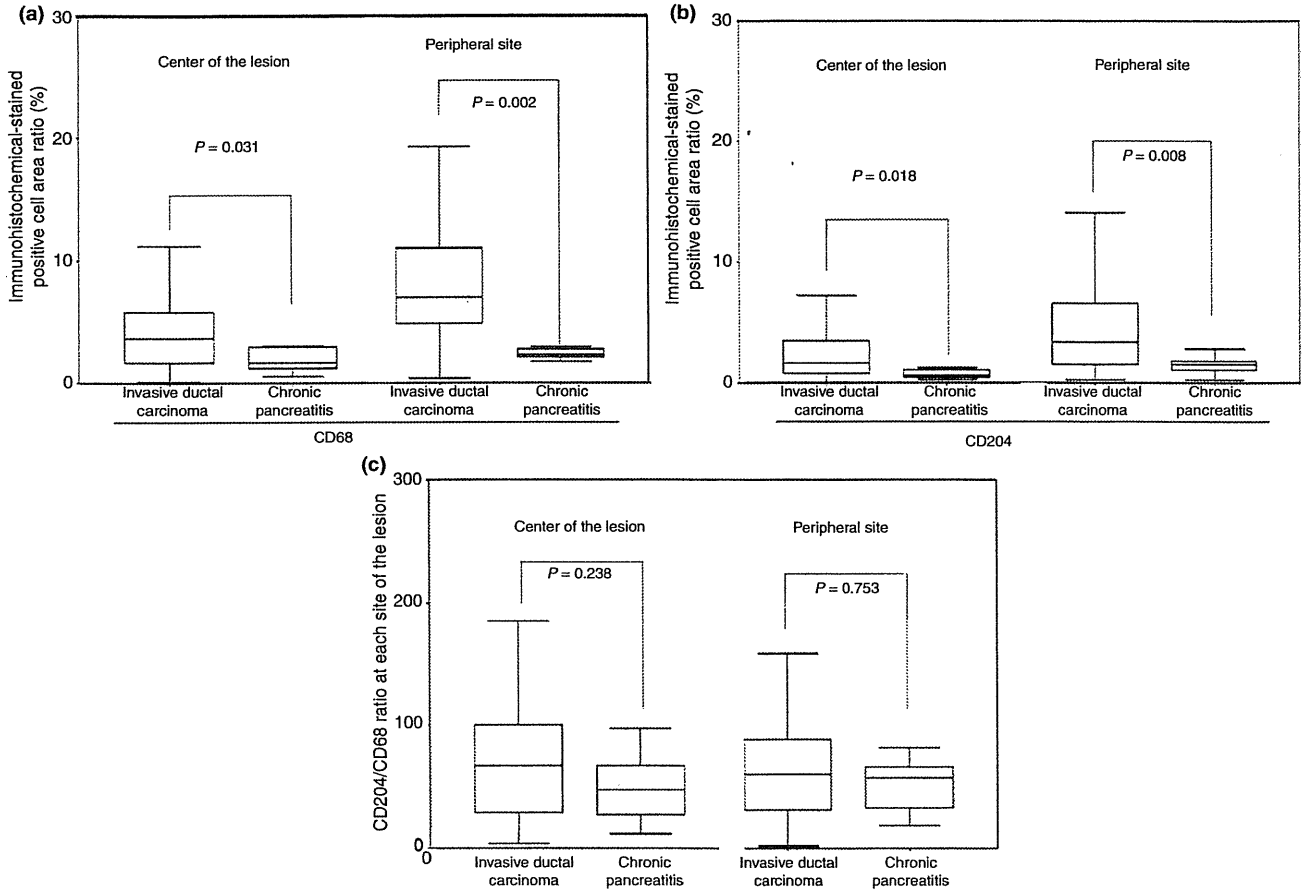

Fig. 2. Distribution of CD68- and CD204-positive cells at the center and periphery of the lesion in invasive ductal carcinoma and chronic pancreatitis. In each site of the lesion and for each immunohistochemical stain, significantly more immunopositive cells were observed in invasive ductal carcinoma than in chronic pancreatitis. (a,b) The CD204/CD68 ratio does not show a significant difference between these two types of lesions (c).

under low-power magnification (×100): 0, no invasion of cancer cells; (i) invasion of a few cancer cells (1–3 points); (ii) moderate invasion of cancer cells (4–8 points); and (iii) marked invasion of multiple cancer cells (>8 points).[30]

The following clinicopathological factors were investigated retrospectively to assess their impact on survival: age ($\leq$64 years *vs* >64 years); sex; serum carcinoembryonic antigen ($\leq$3.5 ng/mL *vs* >3.5 ng/mL), serum carbohydrate antigen 19-9 ($\leq$109 U/mL *vs* >109 U/mL); grade of tumor differentiation (well *vs* moderate or poor); tumor size ($\leq$3 cm *vs* >3 cm); serosal invasion (absent *vs* present); retropancreatic tissue invasion (absent *vs* present); portal vein invasion (absent *vs* present); lymphatic invasion (ly0, 1 *vs* ly2, 3); venous invasion (v0, 1 *vs* v2, 3); intrapancreatic nerve invasion (ne0, 1 *vs* ne2, 3); extrapancreatic nerve plexus invasion (absent *vs* present); and lymph node involvement (absent *vs* present).

Antibodies and immunohistochemistry. Paraffin-embedded blocks of tumor at the maximum diameter were cut into 3-μm serial sections. The sections were deparaffinized in xylene, dehydrated in a graded ethanol series, and immersed in 0.3% hydrogen peroxide in methanol for 15 min to inhibit endogenous peroxidase activity. For antigen retrieval, the slides were heated at 95°C for 15 min in a microwave oven (H2800 Microwave Processor; Energy Beam Sciences, East Granby, CT, USA) in 0.1 M citric acid buffer then allowed to cool for 1 h at room temperature. After washing the slides three times in PBS, non-specific binding was blocked by pre-incubating in 2% normal swine serum in PBS (blocking buffer) for 30 min at room temperature. Individual slides were then incubated overnight at 4°C in mouse anti-human CD68 antibody (1:400 in blocking buffer; Dako, Glostrup, Denmark) or mouse anti-human CD204 antibody (Scavenger Receptor class A-E5, 1:400 in blocking buffer; Transgenic, Kumamoto, Japan). The slides were again washed three times with PBS and incubated with EnVision (Dako) for 1 h at room temperature. After extensive washing with PBS, the color reaction was developed with 2% 3, 3′-diaminobenzidine in 50 mM Tris-buffer (pH 7.6) containing 0.3% hydrogen peroxide. The sections were then counterstained with Mayer's hematoxylin, dehydrated, and mounted.

Definition of center of lesion and peripheral site. To identify the center of the lesion, H&E stained sections were scanned at a magnification of ×40, and the margin of the tumor was marked on each slide. The intersection of the major and minor axes was defined as the center of the lesion, and four fields including the center at a magnification of ×100 were defined as the center of the lesion. Peripheral sites were defined as fields that included cancer cells and adjacent non-cancerous cells at a magnification of ×100. In the pancreatitis cases, the same procedure was used to identify the center and the margin of the dense fibrosing area.

Evaluation of immunohistochemistry (IHC). The IHC-positive cells were quantified by determining the percentage of IHC-positive cells in an area (IHC%) and the IHC-positive cell

doi: 10.1111/j.1349-7006.2012.02411.x

Table 2. Distribution of the percentage of the CD68-positive cell area at the center and periphery of lesions in pancreatic tumors according to clinicopathological features

Parameter	Category	*n*	Central CD68%, median (range)	*P*	Peripheral CD68%, median (range)	*P*
Age (years)	≤64	58	3.75 (0.22–18.60)	0.574	6.25 (0.47–18.70)	0.422
	>64	49	3.63 (0.22–16.40)		7.58 (0.37–25.10)	
Gender	Male	63	3.47 (0.22–18.60)	0.582	6.54 (0.47–25.10)	0.695
	Female	44	3.96 (0.22–17.80)		7.26 (0.37–18.40)	
CEA (ng/mL)	≤3.5	57	3.64 (0.22–11.20)	0.980	6.92 (0.86–25.10)	0.450
	>3.5	50	3.82 (0.22–18.60)		6.76 (0.37–23.20)	
CA19-9 (U/mL)	≤109	53	3.86 (0.22–18.60)	0.815	6.19 (0.47–23.20)	0.108
	>109	54	3.64 (0.42–17.80)		7.50 (0.37–25.20)	
Differentiation	Well	31	3.88 (0.22–0.42)	0.752	7.97 (0.86–23.20)	0.374
	Moderate/Poor	76	3.64 (0.42–18.60)		6.34 (0.37–25.10)	
Tumor size (cm)	≤3.0	66	3.72 (0.22–18.60)	0.414	6.20 (0.69–25.10)	0.526
	>3.0	41	3.25 (0.42–17.80)		7.42 (0.37–18.70)	
Serosal invasion	Absent	84	3.65 (0.22–18.60)	0.554	6.49 (0.37–25.10)	0.451
	Present	23	3.86 (0.22–17.80)		7.42 (0.93–18.40)	
Retroperitoneal invasion	Absent	9	3.86 (0.47–12.10)	0.556	8.39 (0.37–14.30)	0.827
	Present	98	3.65 (0.22–18.60)		6.54 (0.47–25.10)	
Lymphatic invasion	ly0/1	60	3.80 (0.22–18.60)	0.660	6.25 (0.47–23.20)	0.332
	ly2/3	47	3.47 (0.44–17.80)		7.59 (0.37–25.10)	
Vessel invasion	v0/1	10	3.49 (0.64–12.10)	0.822	6.75 (1.56–16.60)	0.756
	v2/3	97	3.65 (0.22–18.60)		6.92 (0.37–25.10)	
Intrapancreatic nerve invasion	ne0/1	27	3.78 (0.22–16.40)	0.917	7.09 (0.93–25.20)	0.346
	ne2/3	80	3.65 (0.22–18.60)		6.92 (0.37–25.10)	
Extrapancreatic nerve	Absent	48	3.72 (0.22–18.60)	0.975	7.06 (0.69–25.10)	0.643
Plexus invasion	Present	59	3.64 (0.22–17.80)		6.22 (0.37–23.20)	
Portal vein invasion	Absent	81	3.56 (0.22–18.60)	0.437	7.37 (0.47–25.10)	0.079
	Present	26	3.92 (0.42–11.00)		5.92 (0.37–15.90)	
Lymph node involvement	Absent	22	1.82 (0.22–11.20)	0.091	5.45 (0.69–19.30)	0.045*
	Present	85	3.78 (0.22–18.60)		7.42 (0.37–25.10)	

*$P < 0.05$. Differences between the two groups were evaluated using the Mann–Whitney *U*-test. CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; ly, lymphatic; ne, intrapancreatic nerve; v, venous.

count, which was generally used to evaluate immunohistochemical staining.

Tumor-associated macrophages identified as CD68- or CD204-positive cells were defined as cells with oval to round nuclei that showed strong membranous/cytoplasmic staining but no nuclear staining. After scanning the immunostained slide at a magnification of ×100, the three areas with the greatest number of macrophages in both the center of the lesion and the peripheral site were selected as hot spots. The Automeasure function in Axio Vision 4.7.1 software (Carl Zeiss, Oberkochen, Germany) was used to distinguish the immunopositive area and to objectively calculate the summed areas of the immunopositive cells in each hot spot at a magnification of ×400. The IHC % (summed area of CD68- or CD204-positive cells/measured area ×100) was then calculated for each site (Fig. 1).

The number of macrophages was counted in three hot spots at ×400 magnification using a micrometer. The mean number of infiltrating macrophages was then calculated.

Statistical analysis. Correlations between IHC% and macrophage count for CD68 and CD204 in the center of the lesion and the peripheral sites were evaluated using Spearman's rank correlation coefficients. Differences in macrophage infiltration between the two groups were evaluated using the Mann–Whitney *U*-test. Overall survival time was calculated from the date of pancreaticoduodenectomy to August 24, 2010. Parameters that were significantly associated with disease-free survival (DFS) or overall survival rates evaluated in univariate analyses using log–rank tests were further analyzed with multivariate analysis using the Cox proportional hazard regression model. Crude overall survival curves were plotted using the Kaplan–Meier method. All *P*-values were two-sided, and the significance level was set at $P < 0.05$. All statistical analyses were carried out using the Statistical Package for the Social Sciences 11.5 J for Windows software (SPSS Inc., Chicago, IL, USA).

Results

Comparison of the area ratio of IHC-positive cells and IHC-positive macrophage count. To validate auto-measurement of IHC-positive cell areas, the correlation between IHC-positive cell numbers and IHC% was examined. The median CD68 count was 21.0 (range, 1.7–64.0) at the center and 42.0 (range, 13.3–94.3) at the periphery of the lesion. The median CD204 count was 14.0 (range, 0.3–48.3) at the center and 24.7 (range, 4.0–75.3) at the periphery. The CD68% and CD204% strongly correlated with the number of CD68- and CD204-positive cells at the center and the periphery of the tumor in pancreatic IDCs ($P < 0.001$, R [correlation coefficient] >0.4). To ensure objectivity, auto-measurement of the IHC% was used to quantify immunoreactivity in this study (Fig. S1).

Distribution of CD68- and CD204-positive cells in pancreatic IDC and CP. A series of 107 IDC specimens of the pancreas and 11 specimens of CP were examined for CD68 and CD204 expression in the center and periphery of the lesion. In the IDC series, the median CD68% was 3.65% (range, 0.05–18.6%) at the center of the lesion and 9.92% (range, 0.37–25.1%) at the periphery, whereas the median CD68% of the CP series was 1.62% (range, 0.55–6.20%) at the center of the lesion and 2.29% (range, 1.13–19.5%) at the periphery ($P = 0.031$ at the center, $P = 0.002$ at the periphery). The median CD204% was 1.64% (range, 0.06–18.1%) at the center of the lesion and

Table 3. Distribution of central and peripheral CD204-positive cell area ratios in pancreatic tumors according to clinicopathological features

Parameter	Category	*n*	Central CD204%, median (range)	*P*	Peripheral CD204%, median (range)	*P*
Age (years)	≤64	58	1.54 (0.06–18.10)	0.970	3.43 (0.34–12.80)	0.846
	>64	49	1.65 (0.22–9.010)		3.27 (0.27–14.00)	
Gender	Male	63	1.51 (0.06–9.310)	0.364	3.27 (0.27–14.00)	0.552
	Female	44	1.77 (0.19–18.10)		3.59 (0.43–14.00)	
CEA (ng/mL)	≤3.5	57	1.45 (0.10–11.90)	0.064	3.31 (0.27–14.00)	0.469
	>3.5	50	2.02 (0.06–18.10)		3.80 (0.34–14.00)	
CA19-9 (U/mL)	≤109	53	1.56 (0.10–9.31)	0.983	3.37 (0.27–14.00)	0.400
	>109	54	1.66 (0.06–18.10)		3.41 (0.34–14.00)	
Differentiation	Well	31	1.38 (0.06–18.10)	0.477	3.43 (0.27–14.00)	0.995
	Moderate/Poor	76	1.69 (0.10–9.31)		3.33 (0.44–14.00)	
Tumor size (cm)	≤3.0	66	1.45 (0.13–9.31)	0.110	3.10 (0.27–14.00)	0.031*
	>3.0	41	2.10 (0.06–18.10)		3.38 (0.34–12.70)	
Serosal invasion	Absent	84	1.66 (0.06–11.90)	0.797	3.34 (0.27–14.00)	0.575
	Present	23	1.34 (0.10–18.10)		4.23 (0.55–12.20)	
Retroperitoneal invasion	Absent	9	1.68 (0.22–8.84)	0.439	6.10 (1.21–11.80)	0.346
	Present	98	1.60 (0.06–18.10)		3.37 (0.27–14.00)	
Lymphatic invasion	ly0/1	60	1.45 (0.22–11.90)	0.201	3.10 (0.27–14.10)	0.151
	ly2/3	47	1.96 (0.06–18.10)		4.26 (0.34–12.80)	
Vessel invasion	v0/1	10	1.17 (0.19–7.98)	0.309	3.33 (0.43–7.18)	0.460
	v2/3	97	1.66 (0.06–18.10)		3.43 (0.27–14.00)	
Intrapancreatic nerve invasion	ne0/1	27	1.64 (0.43–9.31)	0.659	3.38 (0.43–11.80)	0.954
	ne2/3	80	1.64 (0.06–18.10)		3.40 (0.27–14.00)	
Extrapancreatic nerve plexus invasion	Absent	48	1.73 (0.06–11.90)	0.641	3.34 (0.34–14.00)	0.925
	Present	59	1.56 (0.10–18.10)		3.43 (0.27–14.00)	
Portal vein invasion	Absent	81	1.44 (0.06–18.10)	0.012*	3.31 (0.27–14.0)	0.263
	Present	26	2.56 (0.44–9.01)		4.13 (0.55–14.0)	
Lymph node involvement	Absent	22	1.12 (0.13–4.20)	0.018*	0.94 (0.44–8.83)	0.003*
	Present	85	1.86 (0.06–18.10)		4.06 (0.27–14.0)	

*$P < 0.05$. Differences between the two groups were evaluated using the Mann–Whitney *U*-test. CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; ly, lymphatic; ne, intrapancreatic nerve; v, venous.

3.38% (range, 0.27–14.0%) at the periphery in the IDC series, whereas the median CD204% in the CP series was 0.60% (range, 0.26–3.78%) at the center of the lesion and 1.59% (range, 0.32–3.54%) at the periphery ($P = 0.018$ at the center, $P = 0.008$ at the periphery). In each series, CD68- and CD204-positive cells were more frequently observed in the periphery than at the center of the lesions (Fig. 2). The CD204/CD68 ratios at the center and periphery were compared between IDC and CP cases to evaluate the population of cells with the M2 phenotype. In IDC cases, the median CD204/CD68 ratio was 67.6% (range, 3.6–185.4%) at the center of the lesion and 59.9 (range, 2.1–158.5%) at the peripheral sites, whereas the median CD204/CD68 ratio was 47.3% (range, 12.2–96.9%) at the center and 57.6% (range, 18.1–81.7%) at the periphery in CP cases. These differences were not significant ($P = 0.238$ at the center, $P = 0.753$ at the periphery; Fig. 2).

Distribution of CD68% and CD204% according to clinicopathological features. The relationship between clinicopathological features and macrophage infiltration was evaluated using Mann–Whitney *U*-tests (Tables 2, 3). The IDCs with lymph node involvement showed elevated expression of peripheral CD68 ($P = 0.045$), central CD204 ($P = 0.018$), and peripheral CD204 ($P = 0.003$). Cases with tumors >3.0 cm were significantly correlated with high peripheral CD204 expression ($P = 0.031$), and those with portal vein invasion were significantly correlated with high central CD204 expression ($P = 0.012$).

Univariate and multivariate analyses of parameters significantly associated with overall survival and DFS. The median IHC% of infiltrating macrophages was used to divide the cases into two groups, high (above the median value) and low (equal to or below the median value). Univariate analyses using log–rank tests were carried out to compare survival according to IHC% (Table 4), and overall survival curves were obtained with the Kaplan–Meier method (Fig. 3). Univariate analysis (Table 4) produced the following candidates for predicting prognosis: tumor size > 3.0 cm ($P = 0.0001$); lymph node involvement ($P = 0.0106$); lymphatic invasion ($P = 0.0171$); extrapancreatic nerve plexus invasion ($P = 0.0025$); and high central and peripheral CD204 expression ($CD204^{high}$) ($P = 0.0248$ at the center, $P < 0.0001$ at the periphery). Multivariate analysis (Table 5) revealed the following independent prognostic factors: tumor size > 3.0 cm (hazard ratio [HR], 2.017; $P = 0.002$); extrapancreatic nerve plexus invasion (HR, 1.992; $P = 0.002$); and peripheral $CD204^{high}$ (HR, 2.781; $P < 0.001$).

Univariate analysis (Table 4) showed that tumor size > 3.0 cm ($P = 0.0058$), serosal invasion ($P = 0.0427$), extrapancreatic nerve plexus invasion ($P = 0.0057$), and peripheral $CD204^{high}$ ($P = 0.0010$) were correlated with shorter DFS. Multivariate analysis (Table 5) revealed that extrapancreatic nerve plexus invasion (HR, 1.882; $P = 0.008$) and peripheral $CD204^{high}$ (HR, 1.864; $P = 0.010$) were independent risk factors for DFS. Initial recurrent sites of IDC were considered to be liver metastasis ($n = 38$), local recurrence ($n = 38$), or peritoneal dissemination ($n = 20$). The DFS curves for these groups were plotted using the Kaplan–Meier method to determine any significant impact of high CD204 expression at the peripheral site. The peripheral $CD204^{high}$ group had a significantly shorter DFS period than the peripheral $CD204^{low}$ group when stratified by initial liver metastasis and local recurrence (Fig. 4).

doi: 10.1111/j.1349-7006.2012.02411.x

Table 4. Univariate analyses of overall survival (OS) and disease-free survival (DFS) in patients with invasive ductal carcinoma of the pancreas

Factor	Category	*n*	OS, median (range)	*P* (uni)	DFS, median (range)	*P* (uni)
Age (years)	≤64	58	15.0 (3–145)	0.1561	7.5 (2–145)	0.1678
	>64	49	16.0 (1–90)		8.0 (1–90)	
Gender	Female	44	14.0 (1–77)	0.6205	8.0 (1–77)	0.4528
	Male	63	16.0 (2–145)		8.0 (1–145)	
CEA (ng/mL)	≤3.5	57	16.0 (2–145)	0.1757	11.0 (1–145)	0.1374
	>3.5	50	11.5 (1–90)		5.5 (1–90)	
CA19-9 (U/mL)	≤109	53	19.0 (1–90)	0.9288	8.0 (1–90)	0.3710
	>109	54	13.0 (3–145)		7.5 (2–145)	
Differentiation	Well	31	20.0 (1–77)	0.2594	15.0 (1–77)	0.1694
	Moderate/Poor	76	12.5 (2–145)		6.5 (1–145)	
Tumor size (cm)	≤3.0	66	19.0 (2–145)	0.0001*	11.0 (2–145)	0.0058*
	>3.0	41	10.0 (1–52)		6.0 (2–34)	
Serosal invasion	Absent	84	16.0 (1–145)	0.1058	10.0 (1–145)	0.0427*
	Present	23	12.0 (2–39)		6.0 (2–34)	
Retroperitoneal invasion	Absent	9	8.0 (4–53)	0.6294	6.0 (2–53)	0.5389
	Present	98	15.5 (1–145)		8.0 (1–145)	
Portal vein invasion	Absent	81	16.0 (1–90)	0.0745	8.0 (1–90)	0.4140
	Present	26	12.0 (3–145)		6.5 (2–145)	
Lymphatic invasion	0/1	60	20.0 (1–145)	0.0171*	9.5 (1–145)	0.1598
	2/3	47	11.0 (2–63)		6.0 (1–64)	
Vessel invasion	0/1	10	26.0 (6–77)	0.1072	17.0 (3–77)	0.2669
	2/3	97	13.0 (1–145)		8.0 (1–145)	
Intrapancreatic nerve invasion	0/1	27	15.0 (4–145)	0.1198	10.0 (2–145)	0.1001
	2/3	80	15.5 (1–77)		8.0 (1–77)	
Lymph node involvement	Absent	22	26.0 (4–90)	0.0106*	12.0 (1–90)	0.0645
	Present	85	13.0 (1–145)		8.0 (1–145)	
Extrapancreatic nerve plexus invasion	Absent	48	19.0 (3–145)	0.0025*	11.5 (2–145)	0.0057*
	Present	59	12.0 (1–53)		7.0 (1–53)	
CD68% at center	≤3.65%	53	19.0 (2–90)	0.5247	8.0 (1–90)	0.6641
	>3.65%	54	13.0 (1–145)		7.5 (1–145)	
CD68% at periphery	≤6.92%	54	19.0 (3–145)	0.3471	8.0 (2–145)	0.4213
	>6.92%	53	12.0 (1–77)		8.0 (1–77)	
CD204% at center	≤1.64%	54	19.0 (1–90)	0.0248*	8.0 (1–90)	0.6195
	>1.64%	53	11.0 (3–145)		6.0 (1–145)	
CD204% at periphery	≤3.39%	54	21.0 (3–90)	<0.0001*	13.5 (1–90)	0.0010*
	>3.39%	53	10.0 (1–145)		6.0 (1–145)	

*$P < 0.05$. Univariate analysis (uni) was carried out using the log–rank test. CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; CD68%, summed area of CD68-positive cells/measured area ×100; CD204%, summed area of CD204-positive cells/measured area ×100.

Discussion

This was the first study to evaluate the distributions of M2 macrophages (CD204-positive cells) in pancreatic IDC and CP. M2 macrophages preferentially accumulated in peripheral rather than central sites in pancreatic IDC and CP. This finding may indicate that non-cancerous cells play an important role in the recruitment of macrophages and the polarization toward M2 macrophages in pancreatic IDC and CP. In CP, macrophages are recruited using chemoattractants produced by myofibroblasts.[31] Myofibroblasts are considered to be the activated state of pancreatic stellate cells (PSCs), and PSCs are activated by pancreatitis[31] and pancreatic cancer cells.[32] Macrophages in pancreatic IDC may have infiltrated because of chemoattractants produced by myofibroblasts derived from PSCs. The polarization toward M2 macrophages may be responsible for the cells producing IL-4 and IL-10 in both IDC and CP tumors. We considered mast cells and PSCs as candidates. Mast cells accumulate in peripheral areas of IDC[33] and intestinal areas of CP[34] and can produce IL-10.[35] Activated PSCs are abundant in IDC and CP tumors and lead to IL-4 production by T cells.[36] Mast cells and PSCs may play important roles in M2 accumulation in IDC and CP. In this study, most peripheral M2 macrophages in pancreatic IDC were dense along the stroma but not along tumor cells, a finding that may reinforce the above speculation.

Accumulated M2 macrophages in pancreatic IDC were more numerous than in CP. In pancreatic IDC, a large tumor was significantly correlated with dense peripheral M2 macrophages. These results indicate that the tumor volume affects accumulation of M2 macrophages. Recent studies have shown that monocyte recruitment is driven by several chemoattractants such as MIP-2, CCL3, and hypoxia-inducible factor-2α, which are secreted by malignant cells and stromal cells and induced by tumor hypoxia.[26,27,37] Tumor-associated macrophages are recruited to tumors by multiple growth factors and chemokines that are often produced by tumor cells themselves.[38,39] Tumor necrosis is increased in large tumors,[7] and TAMs are attracted to and retained in avascular and necrotic areas where they are exposed to tumor hypoxia.[26,27] Large tumors may increase expression of inflammatory mediators from tumor cells, stroma cells, and tumor hypoxia. Thus, increased tumor volume may promote accumulation of M2 macrophages.

The independent impact of M2 macrophages on survival and time to relapse was first revealed with multivariate analysis in pancreatic IDC. Dense accumulation of peripheral M2 macrophages was established as a good predictive mar-

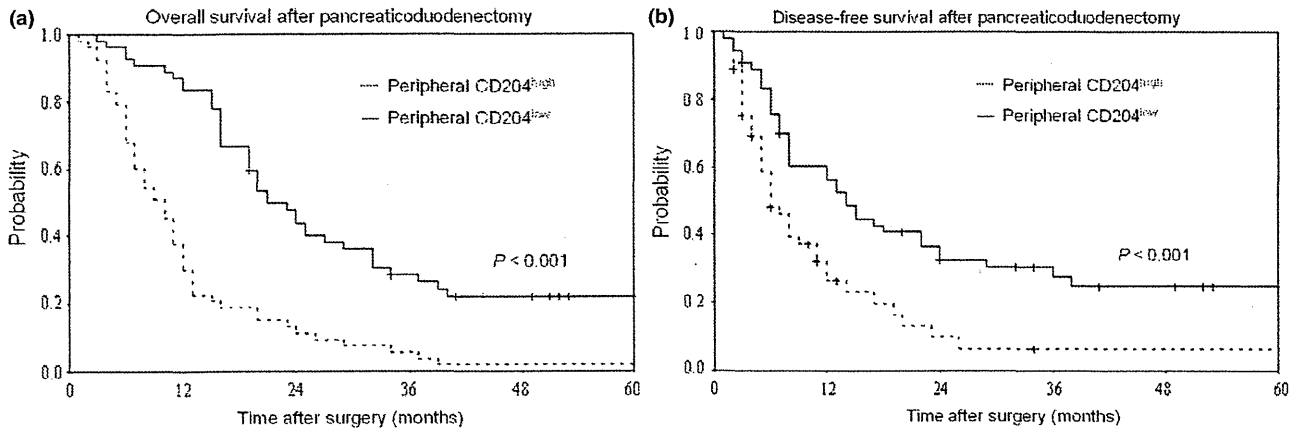

Fig. 3. Overall survival (a) and disease-free survival (b) curves for invasive ductal carcinoma of the pancreas according to the area ratio of peripheral CD204-positive cells. Disease-free survival periods were significantly shorter in patients with peripheral CD204^{high} than in patients with CD204^{low}. Prognosis was significantly worse in patients with peripheral CD204^{high} than for those with CD204^{low}.

ker of survival and recurrence. According to the type of initial recurrence, dense peripheral M2 macrophages were associated with early relapse in liver and the manipulated area of the pancreaticoduodenectomy. This suggests that M2 macrophages may accelerate liver metastasis and local recurrence. Tumor-associated macrophages are important producers of proteases, including MMPs, and of a wide variety of growth factors, such as fibroblast growth factor and epidermal growth factor (EGF) receptor family ligands that can stimulate the growth and motility of tumor cells.[38] Tumor-associated macrophages have been reported to be the most significant source of EGF in tumors,[40] and they are associated with EGF receptor expression and poor outcome in breast cancer.[41] Pollard *et al.* showed that tumor cells respond to macrophage-produced EGF ligands *in vivo* by chemotaxis and invasion, and that macrophages are often associated with vessels.[38,42] Thus, M2 macrophages may provide chemotactic signals that recruit tumor cells to blood vessels and enhance their egress into vasculature, leading to tumor hematogenous metastasis and further local invasion. These effects of M2 macrophages may shorten DFS and overall survival.

Lymph node involvement was significantly correlated with high CD204 expression in peripheral sites of the lesion. Tumor-associated macrophages within the invasive tumor front have a profound influence on the regulation of tumor angiogenesis and lymphangiogenesis by production of vascular endothelial growth factor-C and -D.[9,37,41,43] Elevated lymphangiogenesis by TAMs may promote lymph node metastasis.

The independent prognostic values of large tumor size and extrapancreatic nerve plexus invasion were reported in our previous study[7] and reconfirmed by this study. Time to recurrence was associated with the presence of extrapancreatic nerve plexus invasion. Large tumor size did not show an impact on DFS, because high accumulation of peripheral M2 macrophages correlated with large tumor size.

In conclusion, dense M2 macrophages in peripheral sites were significantly correlated with large tumor size, lymph node involvement, and poor prognosis due to accelerated liver metastasis and local recurrence. The number of accumulated M2 macrophages was associated with tumor volume, but the distribution of M2 macrophages in CP was similar to that in IDC.

Acknowledgments

Supported by Grants-In-Aid for Cancer Research and by a Third Term Comprehensive 10-year Strategy for Cancer Control grant from the Ministry of Health, Labor and Welfare of Japan.

Disclosure Statement

The authors have no conflict of interest.

Table 5. Multivariate analyses of independent significant factors associated with overall survival and disease-free survival in patients with invasive ductal carcinoma of the pancreas

	Overall survival			Disease-free survival		
	HR	95% CI	*P* (multi)	HR	95% CI	*P* (multi)
Tumor size (>3.0 cm)	2.017	1.301–3.127	0.002*	1.492	0.920–2.419	0.105
Serosal invasion present				1.667	0.960–2.896	0.070
Lymph node involvement present	1.112	0.612–2.020	0.727			
Extrapancreatic nerve plexus invasion present	1.992	1.283–3.095	0.002*	1.882	1.176–3.013	0.008*
Central CD204^{high}	1.035	0.673–1.592	0.874			
Peripheral CD204^{high}	2.781	1.740–4.445	<0.001*	1.864	1.164–2.986	0.010*

*$P < 0.05$. Multivariate analyses (multi) were carried out using the Cox regression hazard model. Central CD204^{high}, percentage of CD204-positive cells area over 1.64%; CI, confidence interval; HR, hazard ratio; Peripheral CD204^{high}, percentage of CD204-positive cells area over 3.39%.

doi: 10.1111/j.1349-7006.2012.02411.x

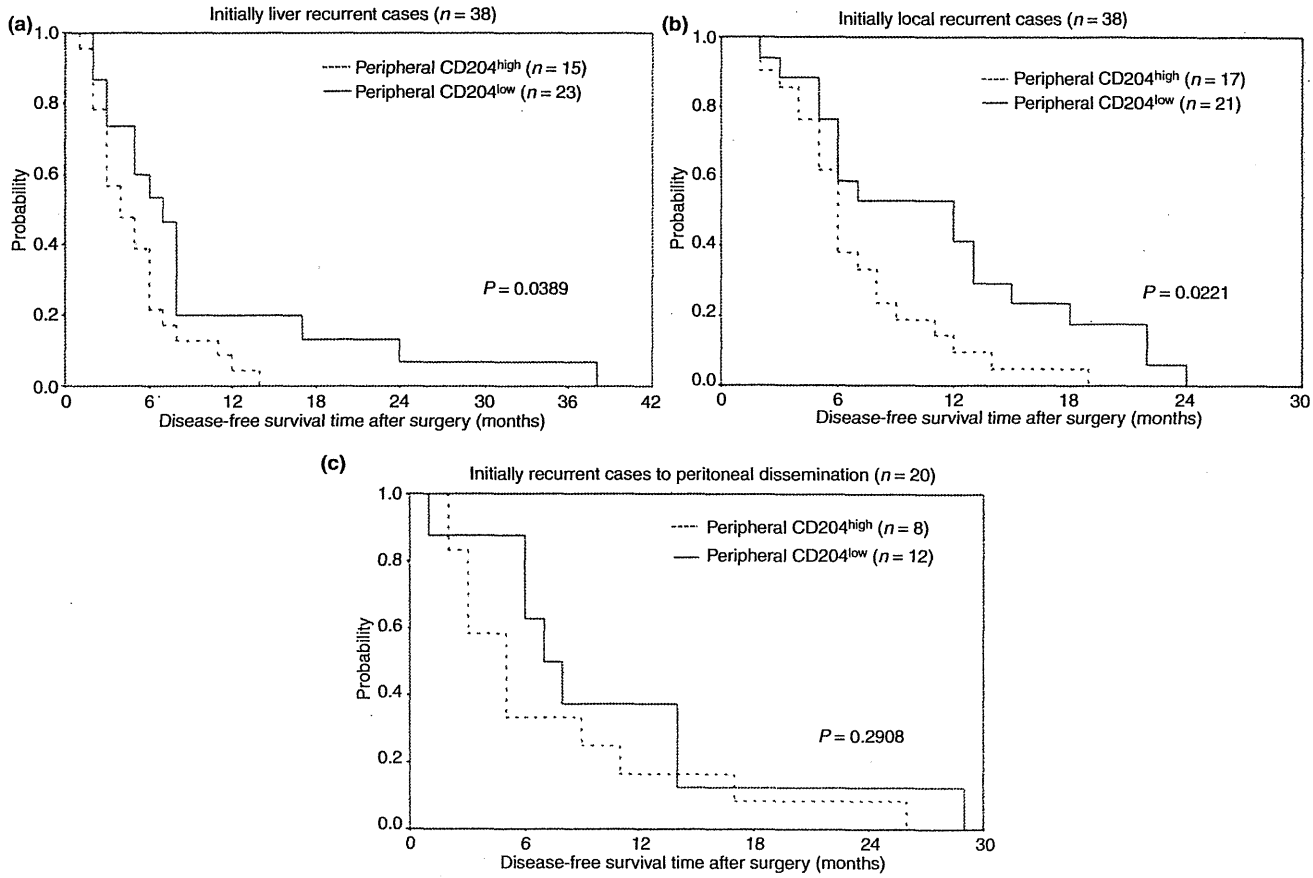

Fig. 4. Disease-free survival curves of invasive ductal carcinoma of the pancreas according to the area ratio of peripheral CD204-positive cells >3.39% and ≤3.39% in three groups that showed initial recurrence in the liver (a), local recurrence (b), and peritoneal dissemination (c). Peripheral CD204^{high} cases showed significantly shorter disease-free survival times in the groups with initial liver metastasis and initial local recurrence.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Correlation between immunopositive cell count and cell area ratio.

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doi: 10.1111/j.1349-7006.2012.02411.x

Surg Endosc (2012) 26:2877–2883
DOI 10.1007/s00464-012-2273-9

Endoscopic balloon dilatation for benign fibrotic strictures after curative nonsurgical treatment for esophageal cancer

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Received: 18 October 2011 / Accepted: 24 March 2012 / Published online: 28 April 2012

Abstract
Background Endoscopic balloon dilatation (EBD) is performed to treat strictures after esophagectomy. However, little is known about using EBD for benign strictures that occur after nonsurgical treatments for esophageal cancer such as chemoradiotherapy (CRT) or endoscopic mucosal resection (EMR). The aim of this study was to evaluate the safety and efficacy of EBD for benign strictures after nonsurgical treatment compared with those after surgery.
Methods We identified 823 patients with esophageal cancer who completed definitive treatments between 2004 and 2007. Of these patients, 122 were enrolled in our study, including 60 who had surgery and 62 who did not have surgery (32 CRT, 30 EMR). The indication criteria for EBD were complaint of dysphagia and the inability to pass a conventional endoscope due to benign stricture. We retrospectively analyzed the safety and efficacy of EBD, and the measured outcomes were treatment success rate, time to treatment success, and refractory stricture rate.
Results Perforation occurred in 3 (0.3 %) of 1,077 EBD sessions, with no bleeding. Efficacy was evaluated in 110 of the 122 patients. While the treatment success rate was over 90 % in both the surgery and the nonsurgery group, there was a significant difference in the median time to treatment success between both groups (2.3 vs. 5.6 months, $p = 0.02$: log-rank test). There was a significant difference in the median time to treatment success between CRT and surgery groups (7.0 months, $p = 0.01$), with no significant difference in the EMR group (4.4 months, $p = 0.85$). A significant difference in the refractory stricture rate was evident between the nonsurgery group (75 %) and the surgery group (45 %, $p < 0.01$).
Conclusion EBD for stricture after nonsurgical treatment of esophageal cancer was safe and effective. However, patients with benign strictures after nonsurgical treatment required significantly longer time to recover from dysphasia compared to those after surgery.

Keywords Esophageal stricture · Balloon dilatation · Esophageal cancer

Various nonsurgical curative treatment modalities for esophageal cancer are increasing. For superficial esophageal cancer, endoscopic mucosal resection (EMR) and endoscopic submucosal resection (ESD) are less invasive curative treatments [1–4]. Chemoradiotherapy (CRT) is a standard nonsurgical treatment for locally advanced esophageal cancer, and salvage EMR or photodynamic therapy (PDT) can be curative for local or regional recurrence after CRT [5–7]. However, dysphagia can be caused by fibrotic stricture, even after nonsurgical treatments, with complication rates of 3.3–40 % after CRT [8, 9] and 6.0–18 % after EMR [4, 10, 11]. Severe dysphagia frequently reduces the quality of life in patients undergoing these nonsurgical treatments despite the achievement of a primary cure under organ preservation.

Esophageal dilatation has been the primary therapy for benign esophageal strictures. Common dilators can be categorized as follows: the simple bougie type (Maloney

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