Cancer Science
Japanese Cancer Association JCA
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Multicenter retrospective analysis of systemic chemotherapy for advanced neuroendocrine carcinoma of the digestive system

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Key words

Cisplatin, digestive system, etoposide, irinotecan, neuroendocrine carcinoma

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Funding information

National Cancer Center Research and Development Fund (23-A-22).

Received February 15, 2014; Revised June 11, 2014; Accepted June 24, 2014

Cancer Sci **105** (2014) 1176–1181

doi: 10.1111/cas.12473

This study analyzed outcomes of systemic chemotherapy for advanced neuroendocrine carcinoma (NEC) of the digestive system. Clinical data from 258 patients with unresectable or recurrent NEC of the gastrointestinal tract (GI) or hepato-biliary-pancreatic system (HBP), who received chemotherapy, were collected from 23 Japanese institutions and analyzed retrospectively. Patients had primary sites in the esophagus (n = 85), stomach (n = 70), small bowel (n = 6), colorectum (n = 31), hepato-biliary system (n = 31) and pancreas (n = 31). Median overall survival (OS) was 13.4 months the esophagus, 13.3 months for the stomach, 29.7 months for the small bowel, 7.6 months for the colorectum, 7.9 months for the hepato-biliary system and 8.5 months for the pancreas. Irinotecan plus cisplatin (IP) and etoposide plus cisplatin (EP) were most commonly selected for GI-NEC and HBP-NEC. For patients treated with IP/EP (n = 160/46), the response rate was 50/28% and median OS was 13.0/7.3 months. Multivariate analysis among patients treated with IP or EP showed that the primary site (GI *vs* HBP; hazard ratio [HR] 0.58, 95% confidence interval [CI] 0.35–0.97) and baseline serum lactate dehydrogenase levels (not elevated *vs* elevated; HR 0.65, 95% CI 0.46–0.94) were independent prognostic factors for OS, while the efficacy of IP was slightly better than for EP (HR 0.80, 95% CI 0.48–1.33; P = 0.389). IP and EP are the most common treatment regimens for NEC of the digestive system. HBP primary sites and elevated lactate dehydrogenase levels are unfavorable prognostic factors for survival. A randomized controlled trial is required to establish the appropriate chemotherapy regimen for advanced NEC of the digestive system. This study was registered at UMIN as trial number 000005176.

Neuroendocrine neoplasms (NEN) are rare tumors that exhibit a variety of morphological, functional and behavioral characteristics.[1] The World Health Organization (WHO) has proposed a grading system for NEN that divides them into three categories based on proliferation as follows: (i) neuroendocrine tumor (NET) (G1) with a mitotic count of <2/10 high power fields (HPF) and/or a Ki-67 index of ≤2%; (ii) NET (G2) with a mitotic count of 2–20/10 HPF and/or a Ki-67 index of 3–20%; and (iii) neuroendocrine carcinoma (NEC) with a mitotic count of >20/10 HPF and/or a Ki-67 index of >20%.[2] Among the three categories, NEC is a poorly differentiated, high-grade malignant tumor, previously termed poorly differentiated neuroendocrine carcinoma (PDNEC), including small-cell carcinoma (SCC) and large-cell NEC. The primary sites of NEC are varied in many organs, with NEC arising in the digestive system accounting for 20–68% of cases with extra-pulmonary NEC.[3–7]

In treating advanced extra-pulmonary NEC, guidelines recommend chemotherapy regimens, which are suitable for small-cell lung carcinoma (SCLC).[8–10] Therefore, platinum-containing regimens, such as etoposide plus cisplatin (EP), are commonly used for NEC arising from the digestive system in clinical practice worldwide and irinotecan plus cisplatin (IP) is commonly adopted in Japan. However, no randomized controlled trial has been conducted previously and retrospective reports have been limited in scope and number.[11–15] Therefore, we conducted a multicenter retrospective study on the outcomes of systemic chemotherapy for advanced NEC of the digestive system to obtain useful information to prepare for a future clinical trial.

Materials and Methods

The selection criteria were as follows: (i) a histologically proven NEC such as PDNEC, SCC, mixed endocrine-exocrine carcinoma with a PDNEC component (MEEC), or a neuroendocrine tumor with a rapidly progressive clinical course (clinically-diagnosed NEC); (ii) a primary tumor arising in the digestive system (gastrointestinal tract [GI] or hepato-biliary-pancreatic system [HBP]); (iii) an unresectable or recurrent disease treated with systemic chemotherapy, which was initiated between April 2000 and March 2011; and (iv) no prior treatment, except for surgical resection. Data were collected from the medical records of patients at 23 institutions in Japan using a standardized data collection form. This study was approved by the institutional review boards of the participating institutions and registered with the UMIN Clinical Trials Registry as UMIN 000005176 (http://www.umin.ac.jp/ctr/).

Responses were evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1. Progression-free survival (PFS) was defined as the time from initiation of chemotherapy to confirmation of disease progression or death due to any cause. Overall survival (OS) was defined as the time from initiation of chemotherapy to death due to any cause. Surviving patients were censored on their last follow-up date. PFS and OS were estimated using the Kaplan–Meier method and compared with the log-rank test. Among the patients treated with EP or IP, multiple variate analysis by Cox proportional hazard models was performed, and the hazard ratio (HR) and the corresponding 95% confidence interval (95% CI) for OS were calculated, using the following seven variables selected based on the results of previous investigations and our clinical experience: age (<60 years/≥60 years), sex (male/female), Eastern Cooperative Oncology Group performance status (0–1/≥2), primary site (GI/HBP), liver metastasis (yes/no), prior surgery (yes/no), baseline serum lactate dehydrogenase (LDH) levels (not elevated/elevated), and first-line chemotherapy regimens (EP/IP). Statistical analysis was performed using SPSS software, version 17.0 (SPSS, Chicago, IL, USA).

Results

Patient characteristics. Figure 1 represents the study population flow chart. A total of 258 patients satisfied the selection criteria. Their characteristics are shown in Table 1. The majority of patients were male (71%) and the most common primary site was the esophagus (33%) followed by the stomach (27%). Most patients both in the GI (84%) and HBP (88%) subgroups had Stage IV or recurrent disease.

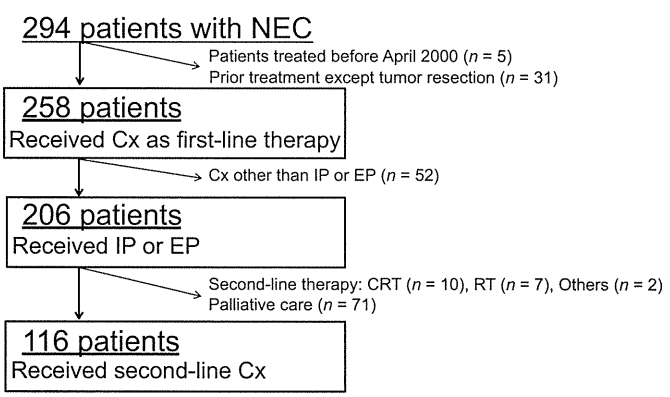

Fig. 1. Flow chart of the study population. CRT, chemoradiotherapy; Cx, chemotherapy; EP, etoposide plus cisplatin; IP, irinotecan plus cisplatin; *n*, number; NEC, neuroendocrine carcinoma; RT, radiotherapy.

Table 1. Patient characteristics

	All patients	GI primary	HBP primary
Number	258	192 (74%)	66 (26%)
Age, years			
Median (range)	62.5 (26–81)	63 (26–81)	58.5 (29–78)
Sex (%)			
Male	182 (71)	153 (80)	29 (44)
Female	76 (29)	39 (20)	37 (56)
Performance status (%)			
0 or 1	240 (93)	176 (92)	64 (97)
≧2	18 (7)	16 (8)	2 (3)
Baseline lactate dehydrogenase (%)			
Elevated	136 (53)	91 (47)	45 (68)
Not elevated	95 (37	79 (41)	16 (24)
No data	27 (10)	22 (11)	5 (8)
Chromogranin A staining (%)			
Positive	172 (67)	122 (64)	50 (76)
Negative	59 (23)	51 (26)	8 (12)
No data	27 (10)	19 (10)	8 (12)
Synaptophysin staining (%)			
Positive	204 (79)	153 (80)	51 (77)
Negative	29 (11)	21 (11)	8 (12)
No data	25 (10)	18 (9)	7 (11)
Ki-67 index (%)			
≥55%	43 (17)	20 (10)	23 (35)
>20%, <55%	27 (10)	18 (9)	9 (14)
No data	188 (73)	154 (80)	34 (52)
Histology (%)			
PDNEC	63 (24)	37 (19)	26 (39)
Small cell carcinoma	122 (47)	99 (52)	23 (35)
MEEC	21 (8)	16 (8)	5 (8)
Clinically diagnosed NEC	52 (20)	40 (21)	12 (18)
Stage (%)			
IV or recurrent	219 (85)	161 (84)	58 (88)
I–III	39 (15)	31 (16)	8 (12)
Primary site (%)			
Esophagus	85 (33)	85 (44)	
Stomach	70 (27)	70 (36)	
Small bowel	6 (2)	6 (3)	
Colorectum	31 (12)	31 (16)	
Hepato-biliary system	31 (12)		31 (47)
Pancreas	35 (14)		35 (53)
Location of metastases (%)			
Liver	136 (53)	95 (49)	41 (62)
Lymph nodes	131 (51)	103 (54)	28 (42)
Lung	27 (10)	25 (13)	2 (3)
Bone	12 (5)	9 (5)	3 (5)
Brain	1 (0.4)	1 (0.5)	0 (0)
Others	30 (11.6)	26 (14)	4 (6)
Prior surgery (+) (%)	76 (29)	66 (34)	10 (15)

GI, gastrointestinal tract; HBP, hepato-biliary-pancretic system; MEEC, mixed endocrine-exocrine carcinoma; NEC, neuroendocrine carcinoma; PDNEC, poorly differentiated neuroendocrine carcinoma.

Treatment. The most common regimen for first-line chemotherapy was IP (*n* = 160, 62%), followed by EP (*n* = 46, 18%) and fluoropyrimidine-based regimens (*n* = 37, 14%), such as 5-fluorouracil/leucovorin/oxaliplatin combination regimen (FOLFOX) and S-1 (Table 2).

Table 2. First-line chemotherapy regimens

	Eso	Stm	SB	CR	HB	P	Total (%)
Number	85	70	6	31	31	35	258 (100)
Irinotecan+Cisplatin (IP)	71	54	2	15	7	11	160 (62)
Irinotecan+Carboplatin	0	0	0	0	1	0	1 (0.4)
Etoposide+Cisplatin (EP)	4	4	2	2	16	18	46 (18)
Etoposide+Carboplatin	2	0	1	1	0	0	4 (2)
Gemcitabine-based†	0	0	0	0	5	5	10 (4)
Fluoropyrimidine-based†	6	11	1	13	3	3	37 (14)
Others	2	1	0	0	0	0	3 (1)

†Overlapped. CR, colorectum; Eso, esophagus; HB, hepato-biliary system; P, pancreas; SB, small bowel; Stm, stomach.

Survival. The median OS of all 258 patients was 11.5 months. In terms of primary site, the median overall was 13.4 months the esophagus, 13.3 months for the stomach, 29.7 months for the small bowel, 7.6 months for the colorectum, 7.9 months for the hepato-biliary system and 8.5 months for the pancreas (Fig. 2). Subgroups were determined by histological analysis and the median OS in months was calculated: PDNEC (12.6, n = 63), SCC (13.0, n = 122), MEEC (12.3, n = 21) and clinically-diagnosed NEC (9.9, n = 52) (Fig. 3). No statistically significant difference in OS was found between the four histology subgroups, including clinically-diagnosed NEC (P = 0.120).

Comparison of irinotecan plus cisplatin and etoposide plus cisplatin regimen efficacy. Among the 258 patients, 206 patients (80%) received either IP or EP as their first-line chemotherapy. Table 3 shows the response rate, median PFS and median OS for these 206 patients. In total, 160 patients who received IP showed a better response rate (50 *vs* 28%, $P < 0.001$), longer PFS (median, 5.2 *vs* 4.0 months, $P = 0.033$) and longer OS (median, 13.0 *vs* 7.3 months, $P < 0.001$) than 46 patients who received EP. According to primary site, 142 patients (89%) in the GI subgroup received IP while 34 patients (65%) received EP in the HBP subgroup. The response rate of IP was significantly better than that for EP in the HBP subgroup (39 *vs* 12%, $P = 0.034$), but there were no statistically significant differences with respect to response rate, PFS, or OS between IP and EP in the GI subgroup.

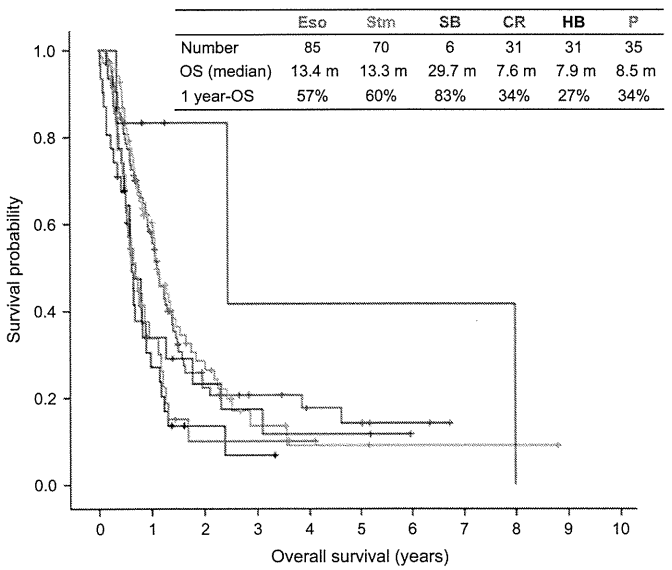

Fig. 2. Kaplan–Meier curves for overall survival according to the primary site. CR, colorectum; Eso, esophagus; HB, hepato-biliary system; OS, overall survival; P, pancreas; SB, small bowel; Stm, stomach.

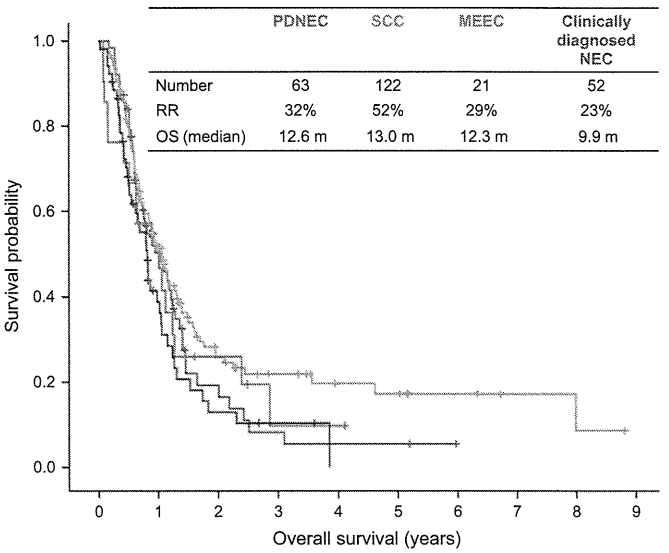

Fig. 3. Kaplan–Meier curves for overall survival according to histology. MEEC, mixed endocrine-exocrine carcinoma; NEC, neuroendocrine carcinoma; OS, overall survival; PDNEC, poorly differentiated neuroendocrine carcinoma; RR, response rate; SCC, small cell carcinoma.

Second-line chemotherapy. Following the failure of IP or EP, 116 patients received second-line chemotherapy. The efficacies of second-line chemotherapy according to the regimen and primary site, GI versus HBP, are shown in Table 4. The efficacy of second-line chemotherapy was slightly better in GI than in HBP patients.

Prognostic factors. In multivariate analysis of prognostic factors for the 206 patients who received EP or IP as their first-line chemotherapy, 23 patients were excluded from the analysis because of no available data on baseline serum LDH

Table 3. Efficacy comparison IP versus EP

	IP	EP	*P*-value
Total			
Number	160	46	
RR	50% (80/160)	28% (13/46)	<0.001†
PFS (median)	5.2 m	4.0 m	0.033‡
OS (median)	13.0 m	7.3 m	<0.001‡
GI			
Number	142	12	
RR	51% (73/142)	75% (9/12)	0.140†
PFS (median)	5.4 m	4.9 m	0.585‡
OS (median)	13.4 m	14.0 m	0.976‡
HBP			
Number	18	34	
RR	39% (7/18)	12% (4/34)	0.034†
PFS (median)	4.4 m	3.7 m	0.056‡
OS (median)	10.1 m	6.9 m	0.050‡

†χ2. ‡Log-rank test. EP, etoposide plus cisplatin; GI, gastrointestinal tract; HBP, hepato-biliary-pancreatic system; IP, irinotecan plus cisplatin; OS, overall survival; PFS, progression-free survival; RR, response rate.

Table 4. Efficacy of second-line chemotherapy

	Number	RR (%)	PFS† (median)	OS‡ (median)
Regimen				
Amrubicin	25	4	1.9 m	8.3 m
EP or CE	23	17	1.9 m	5.0 m
Irinotecan	21	5	2.2 m	5.9 m
S-1	11	27	2.4 m	12.2 m
IP	5	40	4.8 m	8.7 m
Primary site				
GI	87	15	2.3 m	8.1 m
HBP	29	0	1.6 m	5.1 m
Total	116	11	2.1 m	6.3 m

†PFS from second-line chemotherapy. ‡OS from second-line chemotherapy. CE, etoposide plus carboplatin; EP, etoposide plus cisplatin; GI, gastrointestinal tract; HBP, hepato-biliary-pancreatic system; IP, irinotecan plus cisplatin; OS, overall survival; PFS, progression-free survival; RR, response rate.

levels. The primary site (GI *vs* HBP; HR 0.58, 95% CI 0.35–0.97; $P = 0.039$) and baseline serum LDH levels (not elevated *vs* elevated; HR 0.65, 95% CI 0.46–0.94) were independent prognostic factors for OS (Table 5). There was a tendency towards longer survival in patients treated with the IP regimen, although the difference was not statistically significant (IP *vs* EP; HR 0.80, 95% CI 0.48–1.33; $P = 0.389$).

Discussion

In 2013, the NORDIC group reported a large cohort of GI-NEC patients (NORDIC NEC study) and this study is now regarded as an important reference in the NEC field.[16] The current study is also a large-scale study, conducted subsequent to the NORDIC NEC study. Therefore, it is appropriate to compare the major findings of these two recent studies. Both studies indicated that the primary site and baseline serum LDH levels were important prognostic factors. However, survival of pancreatic NEC patients was extremely poor, with a median OS of 8.6 months in our study, compared with the median OS of 15 months in the NORDIC NEC study. This discrepancy could be due to a difference in patient characteristics and/or tumor biology. In our study, 61% of the pancreatic NEC patients had a Ki-67 index ≥55% compared to only 30% for such patients in the NORDIC NEC study. It should be noted, however, that Ki-67 index data were unavailable for almost half (17/35) of the pancreatic NEC patients in our study (data not shown).

Table 5. Univariate and multivariate analysis for overall survival†

	Univariate analysis		Multivariate analysis	
	P-value	HR (95% CI)	*P*-value	HR (95% CI)
Age				
>60 years old (*vs* <60 years old)	0.069	0.73 (0.52–1.03)	0.541	0.89 (0.62–1.28)
Sex				
Female (*vs* male)	0.143	0.76 (0.52–1.10)	0.766	0.94 (0.61–1.43)
Performance status				
0, 1 (*vs* 2≦)	0.022	0.49 (0.26–0.90)	0.130	0.55 (0.26–1.20)
Lactate dehydrogenase				
Not elevated (*vs* elevated)	0.002	0.58 (0.41–0.82)	0.021	0.65 (0.46–0.94)
Primary site				
GI (*vs* HBP)	<0.001	0.48 (0.33–0.70)	0.039	0.58 (0.35–0.97)
Liver metastasis				
(–) (*vs* (+))	0.033	0.69 (0.49–0.97)	0.119	0.76 (0.53–1.08)
First-line chemotherapy				
IP (*vs* EP)	0.001	0.48 (0.33–0.70)	0.389	0.8 (0.48–1.33)
Prior surgery				
(+) (*vs* (–))	0.141	0.71 (0.45–1.12)	0.636	0.89 (0.55–1.45)

†Number = 183 (In analyzing prognostic factors, 23 patients were excluded for whom baseline serum lactate dehydrogenase level data were not available.) CI, confidence interval; EP, etoposide plus cisplatin; GI, gastrointestinal tract; HBP, hepato-biliary-pancreatic system; HR, hazard ratio; IP, irinotecan plus cisplatin.

First-line chemotherapy regimens were different between the two studies. In our study, IP was the most commonly selected regimen, especially for the GI subgroup, while EP was the most commonly selected regimen in the NORDIC NEC study. This discrepancy might be caused by the different recognition of standard regimens of SCLC between Japan and other countries. In terms of treatment for extensive-stage SCLC, IP demonstrated superiority to EP in a randomized controlled trial conducted in Japan (JCOG9511).[17] IP is still considered a standard therapy for extensive-stage SCLC in Japan, although two subsequent randomized controlled trials conducted outside Japan were not able to confirm these earlier results.[18,19] Therefore, it is essential to determine which chemotherapy regimen, IP or EP, is more effective for NEC of the digestive system. However, the number of published reports on chemotherapy for advanced NEC is limited, and most articles investigate a small number of patients, especially for those treated with IP.[11–15] The definition of NEC has also changed recently. Thus, it is difficult to arrive at a current consensus of standard treatment for advanced NEC based on previous reports.

Our study is the largest study to compare the efficacy of EP and IP. The efficacy of IP was slightly better than EP for the treatment of NEC, even after adjusting patient background by multivariate analysis. Although it can be expected that IP might bring more favorable outcomes than EP, especially in the HBP subgroup, there was a considerable confounding bias between chemotherapy regimens and primary sites. Indeed, most patients in the GI subgroup received IP whereas most patients in the HBP subgroup received EP primarily because of different treatment policies among the institutions. Consequently, it remains difficult to determine which regimen was more effective and whether the optimal chemotherapy regimen depends on the primary site for treating advanced NEC based on the results of our retrospective analysis. According to the consensus report of the National Cancer Institute Neuroendocrine Tumor Clinical Trials planning meeting, GI-NET and pancreatic NET should be examined separately in clinical trials.[20] Although, NET and NEC are different disease entities and there is still no consensus with regard to NEC, prognosis was poorer in pancreatic NEC compared with GI-NEC in the current study. Further study is required to determine the appropriateness of treating all digestive NEC with the same chemotherapy regimen, and whether pancreatic NEC should be investigated separately.

Our analysis indicated only a limited efficacy of second-line chemotherapy. Oral topotecan monotherapy has been recommended for patients with platinum refractory or

relapsed SCLC.[8,21–23] Recently, amrubicin was considered a promising regimen in this setting for SCLC, because it significantly improved the response rate compared with topotecan (31 *vs* 17%).[24] Based on these more recent results, amrubicin was the most commonly-used regimen for second-line chemotherapy in our study. However, its response rate and median PFS were only 4% and 1.9 months, respectively. Amrubicin does not appear to be a promising treatment for platinum-refractory NEC. It is also necessary to establish effective treatment in the second-line setting for NEC of the digestive system.

The present study had several limitations. First, there was wide variation in the quality of pathological diagnosis. In the 2010 WHO classification, the importance of the Ki-67 index is emphasized in the grading of NEN. However, Ki-67 index information was not obtained for 73% of the patients in the present study because many of the subjects in this study had been treated before the recent WHO criteria were published in 2010. Recently, histological differentiation has been recognized as important for diagnosis of NEC and it is well known that poor differentiation is related to poor prognosis. Moreover, the present study included clinically-diagnosed NEC patients. In practice, there are some unavoidable cases where tumor grades are estimated according to histological differentiation and tumor growth velocity because adequate specimens are unavailable for histological grading, particularly specimens obtained by endoscopic ultrasound-guided fine-needle aspiration. In the present study, the prognoses of clinically-diagnosed NEC patients were as poor as for patients in the other histology subgroups. This finding may be one rationale for treating clinically-diagnosed NEC patients in accordance with the treatment of histologically-diagnosed NEC patients. Second, we did not collect toxicity data. These limitations can only be resolved by a well-designed prospective clinical trial. We are currently planning a randomized phase III trial comparing IP with EP for the treatment of advanced NEC of the digestive system.

In conclusion, IP and EP are the most commonly selected treatment regimens in Japan for NEC of the digestive system. The primary site and baseline serum LDH levels are independent prognostic factors for NEC, and IP showed a slightly better tendency for efficacy compared to EP. A prospective randomized controlled trial is required to establish the most appropriate chemotherapy regimen for advanced NEC of the digestive system.

Acknowledgments

The authors extend their sincere appreciation to Dr H. Ishii (Cancer Institute Hospital of Japanese Foundation for Cancer Research), Dr T. Yoshino, Dr M. Ikeda, Dr K. Nakachi (National Cancer Center Hospital East), Dr T. Funakoshi (Shizuoka Cancer Center), Dr T Denda, Dr K. Nakamura (Chiba Cancer Center), Dr H. Nishisaki (Hyogo Cancer Center), Dr C. Katada, Dr M. Kida (Kitasato University Hospital), Dr T. Okuno, Dr A. Ikeda, Dr S. Asari (Kobe University Graduate School of Medicine), Dr I. Hyodo, Dr T. Moriwaki (Institute of Clinical Medicine, Tsukuba University Hospital), Dr H. Kawai, Dr S. Hijioka, Dr T. Hasegawa (Aichi Cancer Center), Dr S. Ohkawa, Dr S. Kobayashi (Kanagawa Cancer Center), Dr A. Hosokawa (Toyama University Hospital), Dr S. Tokunaga (Osaka City General Hospital), Dr Y. Kojima, Dr E. Yokota (National Center for Global Health and Medicine), Dr T. Tsuda (St. Marianna University School of Medicine), Dr M. Matsuda, Dr Y Horita (Toyama Prefectural General Hospital), Dr S. Nakazuru (Osaka National Hospital), Dr K. Nakajima (Miyazaki University Hospital), Dr Y. Komatsu, Dr H. Hayashi (Hokkaido University Hospital), Dr K Matsumoto (Faculty of Medicine, Tottori University), Dr T. Ito, Dr Y. Shiina (Kyushu University Hospital), Ms. K. Kondo, Ms. R. Mukouyama and Ms. H. Hosoi (National Cancer Center Hospital).

Disclosure Statement

Junji Furuse serves as a consultant to Bayer, Chugai Pharma, Eisai, Taiho Pharmaceutical, Ono, Zeria, Boehringer Ingelheim and Kyowa Hakko Kirin and received honoraria from Bayer, Lilly, Taiho Pharmaceutical, Chugai Pharma, Novartis and Yakult; Narikazu Boku received a research grant from Taiho Pharmaceutical, Chugai Pharma, Yakult, Ono, Takeda, Bristol-Myers Squibb and Merck Serono and received honoraria from Yakult, Shionogi, Merck Serono, Ono, Taiho Pharmaceutical, Takeda, Chugai Pharma and Diichi-Sankyo; Takuji Okusaka received honoraria from Lilly, Taiho Pharmaceutical, Novartis, Chugai Pharma, Bayer, Pfizer and Sumitomo and received a research grant from Taiho Pharmaceutical, Lilly, Chugai Pharma, Boehringer Ingelheim, Takeda Bio, Yakult, Kyowa Hakko Kirin, Ono, Otsuka, Eisai, Shizuoka Sangyo, Merck Serono, Astra Zeneca and OncoTherapy Science. The study was funded by the National Cancer Center Research and Development Fund (23-A-22); The corresponding author had full access to all of the data in the study and all authors had final responsibility for the decision to submit for publication.

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Zinc finger protein 185 is a liver metastasis-associated factor in colon cancer patients

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Received February 20, 2014; Accepted March 25, 2014

DOI: 10.3892/mco.2014.298

Abstract. LIM domain proteins are involved in several fundamental biological processes, including cell lineage specification, cytoskeleton organization and organ development. Zinc finger protein 185 (ZNF185) is one of the LIM domain proteins considered to be involved in the regulation of cellular differentiation and/or proliferation. However, the detailed functions and properties of ZNF185 in the multistep process of cancer biology have not yet been elucidated. In this study, we analyzed the association between ZNF185 and the clinicopathological characteristics of colon cancer, such as patient age and gender, histological type, lymphatic and venous involvement, T and N status, liver metastasis and stage. ZNF185 protein expression was immunohistochemically analyzed and ZNF185 was detected in the cancer cells of 78 of the 87 colon cancer patients. The correlation between ZNF185 and histological type was significant (P=0.010, G-test). ZNF185 expression was also significantly correlated with liver metastasis (P=0.030, G-test). A multivariate analysis using the Cox proportional hazards model was performed among cause-specific survival rate, ZNF185 expression and clinicopathological characteristics. Histological type, liver metastasis and ZNF185 expression were found to be independent prognostic indicators (P=0.028, P<0.0001 and P=0.036, respectively). Therefore, ZNF185 expression was found to be an independent indicator of liver metastasis and prognosis in patients with colon cancer.

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Key words: zinc finger protein 185, colon cancer, liver metastasis, prognosis

Introduction

Colorectal cancer is among the three leading causes of cancer-related mortality worldwide. Approximately 50% of patients with colon cancer, the predominant type of colorectal cancer, develop liver metastasis, which is considered to be the main cause of death from advanced-stage colon cancer (1). Therefore, it is crucial to elucidate the biological mechanisms underlying liver metastasis of colon cancer and accelerate the development of new treatment strategies.

The liver is the most common site for metastasis from colon and pancreatic cancer (2). Hepatectomy is a potentially curative treatment option for liver metastasis from colon cancer; however, liver metastasis from pancreatic cancer is not considered an indication for surgical treatment (3). Similarities or differences in the biology of liver metastasis between colon and pancreatic cancer remain to be elucidated. We previously established and investigated the highly liver-metastatic human colorectal cancer cell sublines SW48LM2 and LM-BxPC-3, through the serial intrasplenic transfer of hepatic tumor foci formed by parental SW48 colon cancer cells and BxPC-3 pancreatic cancer cells in NOD/Shi-*scid*/IL-2Rγ^{null} mice (4-6). We then performed a quantitative proteome analysis utilizing these established cell lines by our original method (7). The comparison of cellular protein abundance between a pair of 'highly liver-metastatic' cells and its parental cells revealed a series of metastasis-related proteins. In order to identify more universal metastasis-related proteins, we subsequently selected 11 proteins commonly detected among two pairs, i.e., the BxPC-3 and the SW48 subline pairs (unpublished data). These proteins are expected to be good biomarker candidates

and/or plausible causal factors for cancer metastasis. Zinc finger protein 185 (ZNF185) is one of these 11 proteins thus selected.

ZNF185 belongs to the family of LIM domain proteins and contains one LIM zinc-binding domain at the COOH-terminus and an actin-targeting domain (ATD) at the NH_2-terminus. The LIM domain is a cysteine- and histidine-rich double zinc-finger motif named after the three homeodomain proteins: Lin-l 1, Isl-1 and Mec-3 (8-10). The LIM domain is present in a wide range of proteins whose functions include a number of fundamental biological processes, such as cell lineage specification, cytoskeleton organization and organ development (11). Whereas the Zinc finger motif contains the typical DNA binding structures, there is little evidence to support the observation that LIM domains may directly bind DNA (12). LIM domain proteins were found to be distributed in the cytoplasm or nucleus of cells and perform regulatory functions through protein:protein interactions rather than direct interactions with DNA (13,14). ZNF185 is located on chromosome Xq28 and is expressed in the kidney, prostate, pancreas, blood, placenta and ovary, but not in the liver (15). ZNF185 may be involved in regulating cellular differentiation and/or proliferation (16,17). Certain LIM domain-containing proteins were previously shown to be involved in carcinogenic processes (18-27). In basic research on prostate cancer, craniocervical squamous cell carcinoma and non-small-cell lung cancer, the underexpression of ZNF185 mRNA in tumoral tissue was compared to that in matched normal tissue (16,28,29). However, the detailed properties and functions of ZNF185 in the multistep process of tumor invasion have not been investigated in detail (12). The clinical behavior of ZNF185 also remains unknown in relation to the prognosis or treatment of various cancers.

In this study, we investigated the expression level of ZNF185 using immunohistochemistry in 87 cases of colon cancer obtained by complete surgical resection. We also discussed the association between prognosis and the clinical significance of ZNF185 expression.

Materials and methods

Patients. A total of 87 colon cancer specimens were obtained from the surgical specimens of patients with informed consent between April, 2002 and May, 2005. This study has been approved by the Institutional Research Review Board of Tokai University. The tissues were immediately fixed in 40% formaldehyde. The surgical specimens were also processed for routine histopathological analysis.

The patient sample included 48 men and 39 women, with a mean age of 68.30±9.26 years. Well-differentiated adenocarcinomas were found in 60 patients, moderately differentiated adenocarcinomas in 21, poorly differentiated adenocarcinomas in 2 and mucinous adenocarcinomas in 4 patients. The tumors were clinically staged according to the Union for International Cancer Control TNM system. The tumor status was T1 in 5 patients, T2 in 11, T3 in 55 and T4 in 16 patients. A total of 46 patients had lymph node metastasis (N1) and 18 patients had distant metastasis (M1). Lymphatic and venous involvement was found in 74 and 46 patients, respectively. A total of 19 patients had liver metastasis, including 10 synchronous liver metastasis patients. The pathological stages were as follows: stage I, 9 patients; stage II, 30 patients; stage III, 30 patients; and stage IV, 18 patients.

Immunohistochemical (IHC) analysis. Formalin-fixed, paraffin-embedded tissue sections of the tumor samples were analyzed. The paraffin-embedded sections were deparaffinized and stained using the streptavidin-biotin-peroxidase complex method. Rabbit antibodies specific to ZNF185, activated RNA polymerase II transcriptional coactivator p15 (SUB1), β-*N*-acetylhexosaminidase A (HEXA), general transcription initiation factor IIF α subunit (GTF2F1), actinin α 4 (ACTN4) and interleukin enhancer-binding factor 3 (ILF3) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Rabbit anti-glucosidase 2 subunit β (Gluco) and clathrin heavy chain (CLTC) antibodies were purchased from Abcam® (Cambridge, UK). Briefly, the sections were incubated in 0.3% H_2O_2 in methanol, washed in phosphate-buffered saline (PBS) and non-specific protein binding was blocked with normal rabbit serum (Nichirei, Tokyo, Japan). The sections were then incubated overnight in a humid chamber at 4°C, with affinity purified antibodies diluted in PBS, as recommended by the manufacturers. Following three PBS washes, the sections were incubated with peroxidase-labeled polymer conjugated rabbit anti-goat antibody (Histofine Simple Stain Max PO; Nichirei). The amplified immune products were visualized using a 3,3'-diaminobenzidine tetrahydrochloride reaction.

Statistical analysis. Statistical comparisons of data sets were performed by non-parametric analysis using the Mann-Whitney U test. The G-test (likelihood ratio Chi-square test) was applied for comparisons between group frequencies. On multivariate analyses of the cause-specific survival rate, the Cox proportional hazards model was used. Data are presented as means ± standard deviation. The analyses were performed using JMP version 8 software (SAS Institute Inc., Cary, NC, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Identification of ZNF185 as a liver metastasis-associated factor. We selected 8 proteins for the IHC staining experiment among the 11 liver metastasis-associated proteins identified and selected by quantitative proteome studies (unpublished data). The expression of each protein in surgically resected specimens from colon cancer was evaluated by IHC staining. Statistical analyses were performed between expression of the ZNF185, SUB1 and HEXA proteins and liver metastasis in 87 colon cancer cases. A significant correlation was only observed for ZNF185 expression, whereas the correlations were not significant for the expression of SUB1 and HEXA ($P=0.030$, G-test) (Table I). Specific expression of the GTF2F1, ACTN4, ILF3, CLTC and Gluco proteins could not be detected using standard IHC procedures.

ZNF185 expression and clinicopathological characteristics. ZNF185 expression was observed in 78 of the 87 colon cancer cases (Fig. 1). A significant difference was observed between histological type and ZNF185 expression ($P=0.010$,

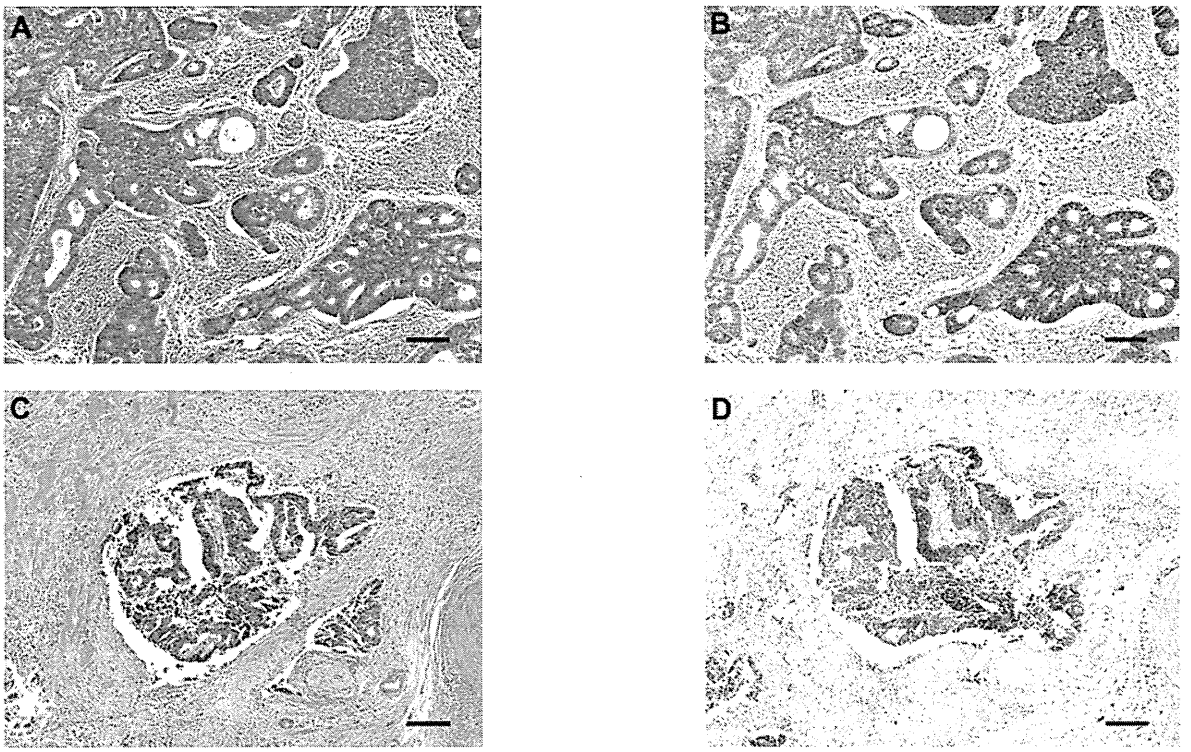

Figure 1. Zinc finger protein 185 (ZNF185) expression in human colon cancer. Colon cancer cells (A and C) exhibit strongly positive cytoplasmic staining for ZNF185 (B and D). Bar, 100 μm.

Table I. Expression of candidate molecules in liver metastasis from colon cancer.

Candidate molecules (n)	Total liver metastasis		P-value
	Positive (19)	Negative (68)	
ZNF185			
Positive (78)	19	59	0.030[a]
Negative (9)	0	9	
HEXA			
Positive (69)	14	55	0.347
Negative (18)	5	13	
SUB1			
Positive (84)	19	65	0.473
Negative (3)	0	3	

G-test; [a]P<0.05. ZNF185, zinc finger protein 185.

G-test). Other clinicopathological correlations, including synchronous liver metastasis, were not significant (Table II). The mean age of ZNF185-positive and -negative patients was 67.67±9.07 and 73.78±9.61 years, respectively (P=0.071, Mann-Whitney U test).

Correlations between prognosis and ZNF185 expression in colon cancer. We analyzed the correlations among cause-specific survival rate, ZNF185 expression and clinicopathological characteristics, such as patient age and gender, histological type, lymphatic and venous involvement, T and N status, synchronous liver metastasis and stage, using the Cox proportional hazards model. The multivariate analyses identified histological type, synchronous liver metastasis and ZNF185 expression as independent prognostic indicators (P=0.029, P<0.0001 and P=0.020, respectively) (Table III).

Discussion

In this study, we identified ZNF185 as a significant liver metastasis-associated factor in colon cancer. To the best of our knowledge, this is the first study to investigate the association between ZNF185 and the clinical characteristics of cancer. ZNF185 expression in colon cancer was found to be an indicator of liver metastasis, as well as an independent prognostic indicator. The histological type and synchronous liver metastasis were found to significantly affect the prognosis of colon cancer patients.

ZNF185 belongs to the LIM domain protein family containing two zinc-finger motifs in the C-terminus, classified as group 3 (12,30). ZNF185 is located on chromosome Xq28 and is expressed in the kidney, prostate, pancreas, blood, placenta and ovary, but not in the liver (15). The complete ZNF185 gene was originally cloned from normal human prostate tissue by Zhang *et al* (17). The expression and localization of ZNF185 in prostate cancer cells and fibroblasts revealed that, in addition to F-actin stress fibers, ZNF185 localized to several other cytoskeleton-related areas, including focal adhesions and filopodia/lamellipodia. ZNF185 was also shown to contain an ATD in the N-terminal region and binds to F-actin directly through the ATD, but not the LIM domains (17). Thus, ZNF185 interacts with F-actin and focal adhesion components. Further studies, focused on identifying proteins interacting with the other domains of

Table II. ZNF185 expression in colon cancer.

Clinicopathological characteristics (n)	ZNF185 expression (+)	(-)	P-value
Gender			
Male (48)	44	4	0.496
Female (39)	34	5	
Histological type			
Well differentiated adenocarcinoma (60)	56	4	0.010[a]
Moderately differentiated adenocarcinoma (21)	18	3	
Poorly differentiated adenocarcinoma (2)	0	2	
Mucinous adenocarcinoma (4)	4	0	
T status			
T1 (5)	5	0	0.599
T2 (11)	9	2	
T3 (55)	50	5	
T4 (16)	14	2	
N status			
N0 (41)	35	6	0.213
N1 (46)	43	3	
M status			
M0 (69)	61	8	0.424
M1 (18)	17	1	
Lymphatic involvement			
Positive (74)	67	7	0.538
Negative (13)	11	2	
Venous involvement			
Positive (46)	41	5	0.835
Negative (41)	37	4	
Synchronous liver metastasis			
Positive (10)	10	0	0.127
Negative (77)	68	9	
Stage			
I (9)	7	2	0.502
II (30)	26	4	
III (30)	28	2	
IV (18)	17	1	

G-test; [a]P<0.05. ZNF185, zinc finger protein 185.

Table III. Multivariate analyses using the Cox proportional hazards model.

Variable	Strata	P-value
Age (years)	68.30±9.26	0.108
Gender	Male, female	0.967
Histological type (adenocarcinoma)	Well, moderate, poorly differentiated, mucinous	0.029[a]
T status	T1, T2, T3, T4	0.087
N status	N0, N1	0.268
Synchronous liver metastasis	Positive, negative	<0.0001[b]
Lymphatic involvement	Positive, negative	0.216
Venous involvement	Positive, negative	0.319
ZNF185 expression	Positive, negative	0.020[a]

[a]P<0.05 and [b]P<0.001. ZNF185, zinc finger protein 185.

ZNF185, may help clarify the mechanism underlying its diverse subcellular localization and function (12). The LIM domains are generally cysteine- and histidine-rich domains, 50-60 amino acids in size, sharing double characteristic zinc finger motifs. A diverse group of proteins containing LIM domains has been identified, which displays various functions, including gene regulation, cell fate determination, tumoral formation and cytoskeleton organization. LIM domain proteins were previously shown to be distributed in the nucleus as well as the cytoplasm and exert their functions through interactions with various protein partners (12). Certain LIM domain proteins are known to play a role in the carcinogenic processes. Epithelial protein lost in neoplasm and testin were found to be downregulated in various cancer cell lines (18,19), whereas LIM domain-only protein 4 is considered to be a negative regulator of breast cancer susceptibility gene 1 and promotes breast tumorigenesis (21,23). LIM and SH3 protein 1 was also identified as a promoter of breast cancer, ovarian cancer and hepatocellular carcinoma and is suggested to be the transcriptional target of p53 (25-27).

ZNF185 gene expression was only shown to be downregulated in matched normal tissues from prostate cancer, craniocervical squamous cell carcinoma and non-small-cell lung cancer (16,17,28,29). Vanaja *et al* (16) reported that the gene expression levels in high-grade (Gleason score 9) prostate cancer cells were suppressed more compared to intermediate grade (Gleason score 6) prostate cancer cells (16). Thus, the dysregulation of ZNF185 gene expression appears to be a frequent event in several cancer types, which suggests its potential role in cancer development. However, there are currently no published reports on ZNF185 in colon cancer. ZNF185 expression was significantly high in well-differentiated adenocarcinoma. In this study, we demonstrated that ZNF185 is a liver metastasis-associated factor, as well as an independent prognosis-deteriorating factor in colon cancer.

Adjuvant chemotherapy is commonly performed to reduce the risk of recurrence and improve the prognosis in patients with colon cancer. According to the National Comprehensive Cancer Network guidelines 2012, all patients with stage III disease should undergo adjuvant chemotherapy. However, stage II patients should also undergo adjuvant chemotherapy when they have high-risk factors, such as T4 lesions, lymphovascular involvement, or poorly differentiated histology (31-33). Liver metastasis is one of the most critical events in the clinical treatment of advanced colon cancer (34). Due to the recent development of clinical studies, certain patients with advanced colon cancer and liver metastasis may become operable. However, the chemotherapeutic regimens for metastatic colon cancer have also improved (35). In our results, ZNF185

indicated liver metastasis with a sensitivity of 100% (19/19) and a specificity of 13% (9/68). These high-sensitivity and low-specificity properties are appropriate for a screening test. There is a possibility that adjuvant chemotherapy may be omitted in ZNF185-negative patients. Therefore, ZNF185 may represent a potential prognostic biomarker of colon cancer.

Cancer cell invasion is a multistep process that includes cell attachment, proteolysis of matrix components and cell migration. Hematogenous liver metastasis, in particular, occurs as a consequence of a well-characterized set of sequential events. The detailed properties and functions of ZNF185 in cancerous invasion have not been fully elucidated. We investigated the clinical significance of ZNF185 in the prognosis and treatment of patients with various types of cancer. The results of the present study, which investigated the behavior of ZNF185 in cancerous invasion, may contribute to the development of novel treatment strategies for advanced colon cancer.

Acknowledgements

The authors would like to thank Tomohisa Machida (Tokai University Hachioji Hospital, Tokyo, Japan) for his technical assistance and helpful discussions.

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Ann Surg Oncol (2014) 21:2882–2888
DOI 10.1245/s10434-014-3701-y

Annals of
SURGICAL ONCOLOGY
OFFICIAL JOURNAL OF THE SOCIETY OF SURGICAL ONCOLOGY

ORIGINAL ARTICLE – PANCREATIC TUMORS

Basing Treatment Strategy for Non-functional Pancreatic Neuroendocrine Tumors on Tumor Size

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ABSTRACT

Background. Surgical resection is advocated for all stages of pancreatic neuroendocrine tumors (PNETs); whether small PNETs can be managed by observation alone is controversial.

Methods. The prognoses of patients with non-functional PNET managed by surgical resection or observation alone were retrospectively analyzed. In patients who had undergone resection, correlation of pathologically assessed tumor extension and grade with tumor size were evaluated.

Results. Nineteen patients with PNET of median tumor diameters of 12 mm (range 6–38 mm) were followed up by observation for 19–162 months. Increase of tumor size >20 % occurred in three patients, resulting in 5-year progression-free survival of 83 %, but no distant metastases occurred. Surgical resection was performed in 71 patients. Tumor size correlated with the incidence of lymph node or hepatic metastases, portal vein invasion, and Ki-67 index. None of the 18 patients with a tumor size ≤15 mm developed lymph node or distant metastases, and all these patients survived without recurrence for 5–283 months. The smallest tumor size with lymph node metastases was 19 mm. The 5-year recurrence-free survivals of patients with a tumor size ≤15 mm (100 %) was significantly better than patients with tumor sizes 16–20 mm (86 %), 21–30 mm (71 %), 31–50 mm (83 %), and >50 mm (48 %).

Conclusion. Because PNETs ≤15 mm in size have little risk of metastases or recurrence, careful observation with serial image studies is acceptable. Once the tumor size exceeds 15 mm, the risk of metastases and recurrence increases significantly.

With ongoing developments in imaging modalities over the last few decades, pancreatic neuroendocrine tumors (PNETs), particularly non-functional ones, have been increasingly recognized. One population-based study showed that the incidence has increased more than twofold in the last 16 years[1] and another showed that the incidence of small non-functional PNETs (≤2 cm) has increased more than sevenfold.[2]

Provided the tumor is considered resectable, the current consensus on optimal treatment for PNETs at any stage is surgical resection, because lymph node metastases can occur even in patients with PNETs of <10–20 mm.[2–9] Several studies have validated aggressive resection of advanced disease with portal vein tumor thrombosis or hepatic metastases.[10–13] For non-functional PNETs, irrespective of tumor size, the National Comprehensive Cancer Network guidelines recommend surgical resection, including regional lymph nodes. They state that enucleation or observation are options for small tumors (<10 mm); however, their criteria are unclear.[14] Of note, few studies have reported long-term outcomes of patients with small PNETs managed by careful observation, provided serial imaging shows no or minimal growth.[15,16] Thus, the optimal strategy for small PNETs is controversial.

In our institution, the management policy for patients with PNETs radiologically assessed as ≤10 mm is close observation at 6-monthly intervals.

This study aimed to evaluate whether tumor size reliably predicts the degree of malignancy and can determine treatment strategy for PNETs.

First Received: 4 February 2014;
Published Online: 18 April 2014

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PATIENTS AND METHODS

Patients

A prospectively collected institutional database of patients with pancreatic tumors was reviewed to identify those treated for non-functional PNET by either surgical resection or observation in our institution from October 1981 to September 2013. The diagnosis of PNET was confirmed pathologically in all patients who had undergone surgical resection, whereas in patients whose tumors had not been resected, the diagnosis of PNET was made by Doppler or contrast enhanced ultrasonography (US) and computed tomography (CT). Fine-needle aspiration (FNA) biopsy was not routinely performed.

Treatment Strategies

Our indication for surgical resection for non-functional PNETs is tumor size >10 mm. Small tumors of up to 10 mm are managed by close observation. Because a few patients with PNET >10 mm refused surgical resection, several patients with larger tumors were treated by observation.

The standard surgical procedure was either pancreaticoduodenectomy or distal pancreatectomy with regional lymph node dissection. For small tumors, parenchyma-preserving procedures were considered. For extensive tumors, portal vein resection or total pancreatectomy was considered. For synchronous liver metastases, simultaneous or secondary hepatic resection was performed provided the tumor was considered resectable.

No patient received preoperative or adjuvant chemotherapy. Every patient was followed-up at 6-monthly intervals by US or CT to evaluate recurrence or tumor progression.

Analyses

In patients undergoing observation, initial tumor size and progression-free survival were evaluated. According to response evaluation criteria in solid tumors,[17] tumor progression was defined as an increase of more than 20 % in diameter. In patients who had undergone surgical resection, first, correlations between tumor size and World Health Organization (WHO) grade based on the Ki-67 index,[18] portal vein invasion, lymph node metastases, and hepatic metastases were analyzed. Distribution by stage based on the European Neuroendocrine Tumor Society (ENETS) TNM classification system[19] was also evaluated. Incidence of postoperative morbidity and subsequent complications were assessed. The severity of pancreatic fistula was classified according to the International Study Group of Pancreatic Fistula criteria.[20] In addition, postoperative recurrence-free survival (RFS) according to tumor size and lymph node status was evaluated.

TABLE 1 Profiles and tumor characteristics of patients in the resection and observation groups

Group	Resection ($n = 71$)	Observation ($n = 19$)	p-Value
Age [years; median (range)]	56 (17–89)	62 (27–79)	0.04
Gender (male/female)	32/39	8/11	0.77
VHL [n (%)]	1 (1)	1 (5)	0.38
Tumor size [mm; median (range)]	28 (0–140)	12 (6–33)	<0.01
Arterial enhancement by CT [n (%)]	64 (90)	19 (100)	0.34

All the above data were obtained at the time of initial diagnosis. Tumor size was based on radiological imaging

VHL Von Hippel–Lindau disease, *CT* computed tomography

Statistical Analysis

Continuous data are expressed as the median and range and were assessed by the Mann–Whitney U test or Kruskal–Wallis test. Categorical data were compared by Pearson's χ^2 test or Fisher's exact test as appropriate. Survival curves were constructed by the Kaplan–Meier method and compared by the log-rank test. A p-value less than 0.05 was considered to be statistically significant in all analyses.

RESULTS

Patient Profiles

During the study period, 81 patients underwent surgical resection of PNETs in our institution. Six patients with functional PNET (gastrinoma, 3; insulinoma, 3), three with mixed acinar-endocrine carcinoma, and one with coexisting invasive ductal carcinoma were excluded. The remaining 71 patients were evaluated as the resection group. Their median age at the time of surgery was 56 years (range 17–81); 39 of these patients (55 %) were female. The observation group consisted of 19 patients, whose median age at the time of referral was 62 years (range 32–79); 11 of these (58 %) were female. The resection and observation groups were completely separate. One patient in the resection group had undergone surgery following 5-year observation at another hospital for a PNET that was initially 10 mm in size and had increased to 21 mm. In the remaining 70 patients, the median interval between the date of diagnosis and surgery was 77 days. Profiles of the patients in each group are summarized in

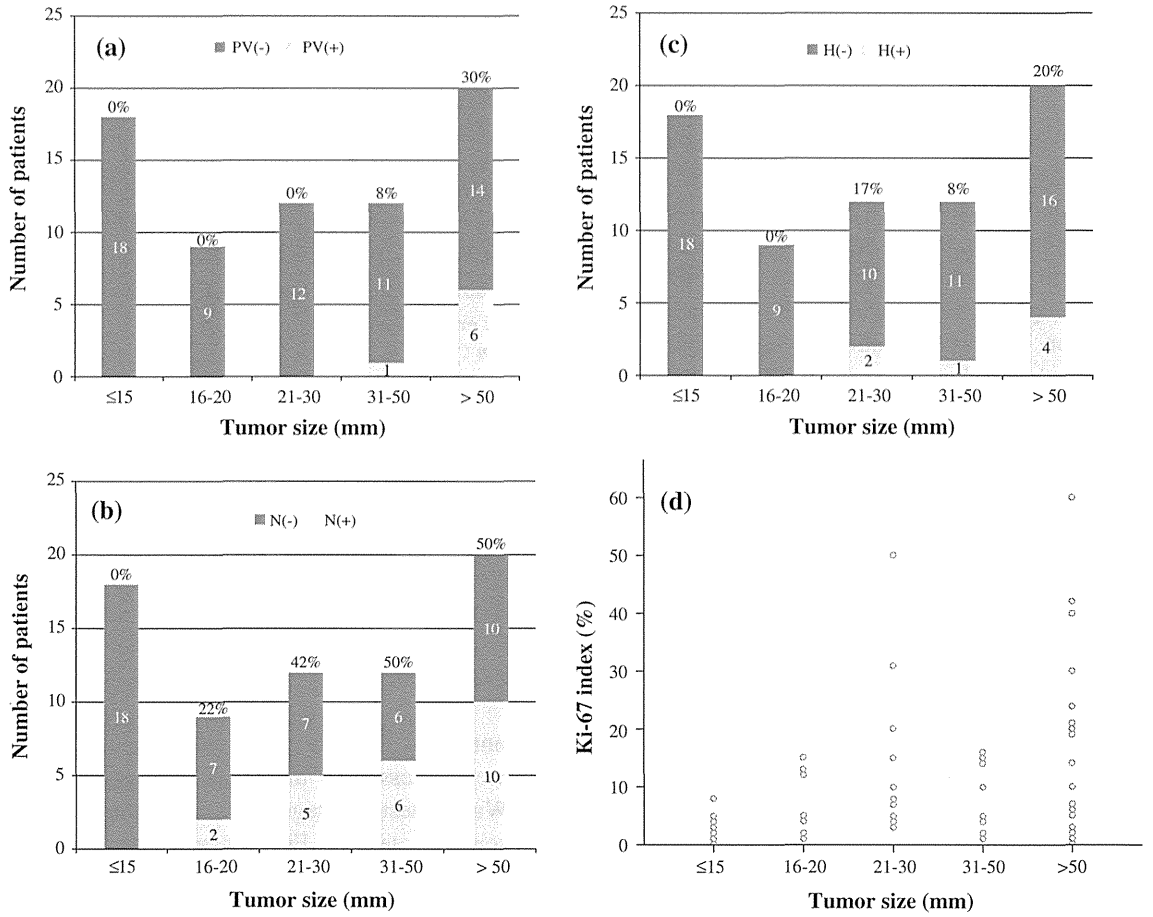

FIG. 1 Incidence according to tumor size of **a** portal vein invasion; **b** lymph node metastases; **c** hepatic metastases. **d** Ki-67 index versus tumor size. The digits above each bar show the positive rates for PV (+), N (+), or H (+). *PV* portal vein invasion, *N* lymph node metastases, *H* hepatic metastases

Table 1. There were two patients with Von Hippel–Lindau disease. All patients had solitary tumors. Tumor size based on CT was significantly larger in the resection group. All tumors in the observation group showed arterial enhancement on CT, but did not in seven patients (10 %) in the resection group. In fact, a definite preoperative diagnosis of PNET was not made in 16 patients in the resection group. The tumors were difficult to differentiate from invasive ductal cancer in eight cases, solid and pseudopapillary neoplasms in four cases, and other tumors (mucinous cystic neoplasm, intraductal papillary mucinous neoplasm, retroperitoneal tumor, metastasis of hepatocellular carcinoma) in four cases.

Prognosis of Patients in the Observation Group

The main reason for observation in patients with tumors of >10 mm was the patients' request. During the median observation period of 45 months (range 19–162), no distant metastases appeared. In any serial image, enlarged lymph node indicating metastasis was not recognized. However, the tumors of three patients increased in size: from 9 to 12 mm in 32 months in one patient, from 10 to 16 mm in 88 months in the second patient, and from 19 to 28 mm in 106 months in the third. Because all three patients refused to undergo surgery, observation was continued. Five-year progression-free survival was 83 %.

Pathologic Findings in the Resection Group

Types of surgical resection were pancreaticoduodenectomy in 31 patients (44 %), distal pancreatectomy in 24 patients (34 %), middle pancreatectomy in 8 patients (11 %), partial resection or enucleation in 5 patients (7 %), and total pancreatectomy in 3 patients (4 %). The total pancreatectomy was performed due to extensive tumor spread along the main pancreatic duct. Seven patients (10 %) had synchronous hepatic metastases, which were resected simultaneously in six patients. In the remaining patient, secondary hepatic resection was planned, but bone metastases were found, prompting abandonment of curative resection.

TABLE 2 Distribution of patients in each ENETS stage according to tumor size

Tumor size (mm)	ENETS stage (%)						Total
	I	IIA	IIB	IIIA	IIIB	IV	
≤15	18 (100)	0	0	0	0	0	18
16–20	3 (33)	4 (44)	0	0	2 (22)	0	9
21–30	0	6 (50)	0	0	3 (25)	3 (25)	12
31–50	0	1 (8)	4 (33)	1 (8)	4 (33)	2 (17)	12
>50	0	0	7 (35)	2 (10)	5 (25)	6 (30)	20
Total	21	11	11	3	14	11	71

ENETS European Neuroendocrine Tumor Society

Figure 1 shows the incidence of portal vein invasion, lymph node metastases, hepatic metastases, and Ki-67 index according to tumor size. There was a tendency for these to be correlated with tumor size. None of the patients with tumors ≤15, ≤20, and ≤30 mm had lymph node metastases, hepatic metastases, and portal vein invasion, respectively. The smallest tumor with lymph node metastasis was 19 mm. All tumors that were WHO grade 3 (corresponding to Ki-67 index >20 %) were larger than 20 mm and the Ki-67 index of all patients with tumors ≤15 mm was less than 10 %.

The numbers of dissected lymph nodes in patients with a tumor size ≤15, 16–20, 21–30, 31–50, and >50 mm were 23 (0–51), 15 (1–37), 17 (1–56), 25 (6–63), and 26 (4–78), respectively, and were comparable among the five groups ($p = 0.37$). No lymph node sampling was performed in four patients, two of whom underwent middle pancreatectomy, one partial resection, and one enucleation for 8–15 mm tumors. The numbers of metastatic lymph nodes in the five groups listed above were 0, 0 (0–2), 0 (0–10), 1 (0–6), and 1 (0–34), respectively. There was a significant difference in the number of metastatic lymph nodes between patients with a tumor size ≤15 and >15 mm ($p < 0.01$), whereas there was no significant difference among the four groups of patients with a tumor size >15 mm ($p = 0.33$). In 64 patients without hepatic metastases, the incidence of lymph node metastases in the five groups was 0/18 (0 %), 2/9 (2 %), 4/10 (40 %), 5/11 (46 %), 7/16 (44 %), respectively. Table 2 shows the distribution of patients of each ENETS stage according to tumor size. All patients with tumors ≤15 mm were classified as stage I, whereas two-thirds of those with PNETs of 16–20 mm were classified as stage II or III.

Postoperative Outcomes and Long-Term Prognosis

Postoperative morbidity occurred in 56 patients (80 %), which was mostly pancreatic fistula in 46 patients (Grade A, 13; Grade B, 32; Grade C, 1[20]) followed by delayed gastric emptying in ten patients. Most of these patients improved conservatively, but arterial bleeding treated by transarterial embolization occurred in one patient. There was no mortality. The median length of stay after surgery was 24 days (12–71). Subsequent complications occurred in 12 patients (17 %) as follows: development of diabetes mellitus, 7 (excluding patients with total pancreatectomy); gastrointestinal bleeding from the anastomotic ulcer, 2; stricture of hepaticojejunostomy required re-anastomosis, 1; stricture of pancreaticojejunostomy, 1; ileus, 1; diarrhea, 1.

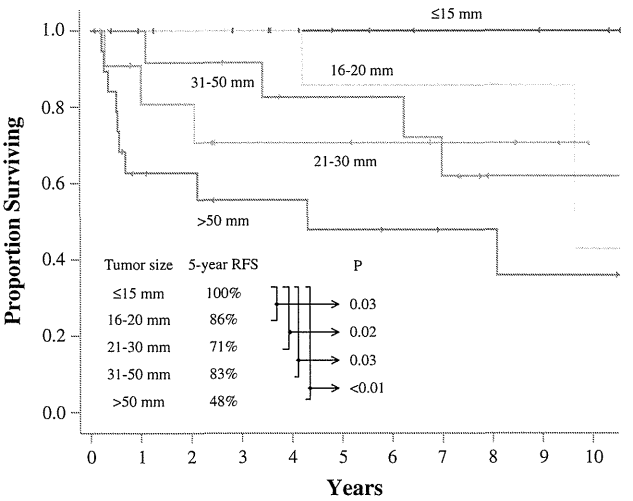

FIG. 2 Postoperative RFS curves according to tumor size. The RFS of patients with tumors ≤15 mm was significantly better than that of any of the other four groups of patients. Differences in RFS among the four groups with tumors >15 mm were not significant ($p = 0.19$). *RFS* recurrence-free survival

During the median postoperative follow-up of 69 months, tumor recurrence was recognized in 19 patients. The site of initial tumor recurrence was most frequently the liver in 14 patients, followed by lymph nodes in two patients, remnant pancreas in one, and bone in one. One had simultaneous liver and lymph node recurrences.

Cumulative 1-, 3-, 5-, and 10-year RFS rates of all 71 patients were 88, 82, 76, and 62 %, respectively. Figure 2 shows the postoperative RFS curves according to tumor size. No patient with tumors ≤15 mm developed recurrence and the RFS for this group was significantly better than for any of the other four groups. The RFS of the four groups of patients with tumors >15 mm did not differ significantly ($p = 0.19$).

DISCUSSION

The present study suggested that small PNETs of up to 10 mm in diameter can be safely observed by serial imaging studies, although the proper size threshold allowing observation could not be determined. It also suggested that the risk of recurrence after surgical resection was significantly lower for PNETs of ≤15 mm than for

TABLE 3 Previous studies concerning lymph node metastases and/or postoperative long-term prognosis of small non-functional PNETs

Authors	Year	N	Tumor size vs. lymph node metastases rate (%)	Prognosis	
				Outcome	Results
Nomura et al.[4]	2009	17	7–15 mm, 0/5 (0) 16–20 mm, 1/2 (50) >20 mm, 5/10 (50)	Postoperative recurrence	7–40 mm (N = 9), 0 for 8–120 months >40 mm (N = 7), 4 (57 %)
Tsutsumi et al.[7]	2012	59[a]	<10 mm, 0/4 (0) 10–14 mm, 0/18 (0) 15–19 mm, 2/13 (15) 20–29 mm, 1/11 (9) ≥30 mm, 6/13 (46)	NA	
Kim et al.[9]	2012	125	≤10 mm, 0/14 (0) ≤20 mm, 1/51 (2)	Postoperative recurrence	≤10 mm (N = 14), 0 ≤20 mm (N = 51), 3 (6 %)
Kuo et al.[2]	2013	1371[b]	1–5 mm, 3/12 (25) 6–10 mm, 5/30 (17) 11–15 mm, 14/65 (22) 16–20 mm, 29/80 (36) >20 mm, 494/913 (54)	10-year DSS	1–5 mm (N = 16), 100 % 6–10 mm (N = 51), 95 % 11–15 mm (N = 94), 76 % 16–20 mm (N = 99), 76 % >20 mm (N = 1108), 59 %
Our study	2014	71	≤15 mm, 0/18 (0) 16–20 mm, 2/9 (22) 21–30 mm, 5/12 (42) 31–50, 6/12 (50) >50 mm, 10/20 (50)	5-year RFS	≤15 mm (N = 18), 100 % 16–20 mm (N = 9), 86 % 21–30 mm (N = 12), 71 % 31–50 mm (N = 12), 83 % >50 mm (N = 20), 48 %

PNET pancreatic neuroendocrine tumor, *DSS* disease-specific survival, *RFS* recurrence-free survival, *NA* not available

[a] The data excluded patients with gastrinoma but included patients with insulinoma, glucagonoma, and somatostatinoma

[b] Population-level study. Others were all single institutional studies

those >15 mm, for whom the tumor was frequently staged as II or more and the risk of metastases was higher. Not only tumor size, but also tumor differentiation grade, Ki-67 index, and lymph node metastases determine the degree of malignancy and long-term prognosis.[3,6,8,21–24] These studies found that differentiation grade and metastatic status, rather than tumor size, were predictors of prognosis. However, tumor size is usually the only one of these factors that can be assessed by preoperative imaging studies. Although several histopathological studies suggested that intratumoral low microvascular density was associated with poor prognosis,[25,26] arterial tumor enhancement by CT did not discriminate RFS in our series (data were not shown in the results).

So far, no standard strategy for small, non-functional PNETs in particular has been established. Most studies advocate surgical resection for PNETs of any size because even small tumors can be malignant or metastasize to lymph nodes.[2–9,27] Previous studies showing the incidence of lymph node metastases and/or prognosis according to tumor size were summarized in Table 3. Lymph node metastases were recognized even with PNETs of <10 mm. There is a possibility, however, that the incidence of lymph node metastases in patients with small PNETs were overestimated due to the following reasons. First, several studies included functional PNETs such as gastrinoma that frequently involve lymph nodes even with image-negative tiny tumors.[28] In the study by Tsutumi et al., the two patients with node-positive PNET of <10 mm were both gastrinomas.[7] Second, lymph node sampling was not performed in all of the patients. Parekh et al.[27] examined the lymph node status of 149 patients who underwent surgical resection and showed that no lymph nodes were identified in the resected surgical specimens in 43 % of the patients. In our series, the number of lymph nodes sampled did not differ according to the tumor size.

To the best of our knowledge, this is the third reported study (the other two being those of Lee et al.[15] and Gaujoux et al.[16].) that has reported long-term outcomes of patients managed without resection. Although there is a possibility that the diagnosis of PNET in the observation group was inaccurate because no histological confirmation was obtained, not all patients underwent biopsy either in the series by Lee et al. or Gaujoux et al. Our study was similar to theirs in that median tumor size with observation was around 10 mm and tumor

size did not change in most patients throughout the follow-up period. Although the risk of metastases was small, there were some malignant PNETs, especially WHO grade 2 tumors, among tumors ≤15 mm in our resection group; this is in accord with the findings of several previous studies.[2,3,5,9,15] Furthermore, the tumors of several patients in the observation group slowly enlarged; however, no patients in this group underwent resection, even when their tumors had increased in size, because they elected to continue observation. Because none of the 19 patients in the observation group underwent biopsy, the Ki-67 indexes of these patients could not be determined. However, the Ki-67 indexes of the tumors ≤15 mm in the resection group were all less than 10 %. Additionally, the tumor of the one patient who underwent resection because follow-up serial imaging studies showed gradual increase in tumor size had a Ki67-index as low as 3 %. Kim et al.[9] reported that 14 PNETs of ≤10 mm were all WHO grade 1. Lee et al.[15] reported that the Ki-67 indexes of all incidentally identified non-functional PNETs were <5 %, although Ki-67 indexes were available in only 44 % of patients. These results suggest that small tumors of up to around 10 mm are generally low grade.

Considering the risks of pancreatectomy is also important. Usually the pancreas with PNET is soft, associating with high risk of pancreatic fistula, as was suggested from the present results. We further showed the incidence of late-onset complications as 17 %. Previous studies showed the incidence of new-onset diabetes after distal pancreatectomy as 9–36 %.[29,30] Therefore, ideally, pancreatic resection should only be performed in patients with malignant PNETs. Evaluation by FNA may be useful. However, insufficient samples, especially with small tumors,[31] or an adverse event such as pancreatitis,[32] precluded us from routinely performing FNA in all patients with pancreatic tumors, and let us advocate the observation of well-enhanced pancreatic small tumor, although the radiological diagnosis of PNET may be inaccurate.

Limitations of this study include that it was a retrospective study of a small number of patients in both the resection and observation groups. Although, in our series, no metastatic disease or postoperative recurrence occurred in patients with PNETs of ≤15 mm, this group included only 18 patients. To review the previous reports, PNETs less than around 10 mm seem to have a rare risk of recurrence (Table 3). More studies with larger series are required to determine a safe cutoff size for non-operative treatment.

CONCLUSION

Small PNETs of up to around 10 mm can be followed up by careful observation with little risk of tumor progression, while once the tumor size exceeds 15 mm, the risk of metastases and recurrence increase.

ACKNOWLEDGMENT This work was supported by a Grant-in-Aid for Young Scientists (B) [#23791518] from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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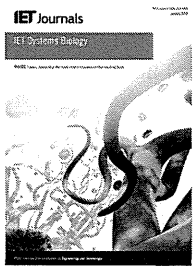
Published in IET Systems Biology
Received on 24th October 2013
Revised on 17th December 2013
Accepted on 23rd December 2013
doi: 10.1049/iet-syb.2013.0044



ISSN 1751-8849

Knowledge discovery for pancreatic cancer using inductive logic programming

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Abstract: Pancreatic cancer is a devastating disease and predicting the status of the patients becomes an important and urgent issue. The authors explore the applicability of inductive logic programming (ILP) method in the disease and show that the accumulated clinical laboratory data can be used to predict disease characteristics, and this will contribute to the selection of therapeutic modalities of pancreatic cancer. The availability of a large amount of clinical laboratory data provides clues to aid in the knowledge discovery of diseases. In predicting the differentiation of tumour and the status of lymph node metastasis in pancreatic cancer, using the ILP model, three rules are developed that are consistent with descriptions in the literature. The rules that are identified are useful to detect the differentiation of tumour and the status of lymph node metastasis in pancreatic cancer and therefore contributed significantly to the decision of therapeutic strategies. In addition, the proposed method is compared with the other typical classification techniques and the results further confirm the superiority and merit of the proposed method.

1 Introduction

In recent years, pancreatic cancer has become an exceptionally devastating disease with surgery as the only treatment with curative intent. It is reported that five-year survival rate, even after tumour resection therapy, is around 10–20% (see, e.g. [1–3]). There is an urgent need for early diagnosis and treatment of pancreatic cancer to further improve the survival rate. Most statistical studies focus on predicting patient survival by analysing relationships between newly developed or found biomarkers and clinicopathological data [4]. However, it is quite difficult to produce a practical biomarker by such kinds of studies. This is mainly due to the fact that patient survival rate is not a simple issue, but related to various factors, such as genetic background of the patient, the nature of the tumour, the age of the patient and so on. Here, we focus on the histological background of tumour itself rather than the patient survival [5]. In many studies, it has been pointed out that tumour characteristics themselves are closely related to the patient survival rate (see, e.g. [6–9]). Once we determine the tumour characteristics of pancreatic cancer, we may design effective and personalised therapeutics before treatment. Furthermore, there is another reason to focus on the tumour differentiation and lymph node metastasis. Note that the current available therapeutic strategies for the pancreatic cancer are chemo or chemo-radiation. Such therapies after surgical resection of pancreatic cancer produce physical harm to patients and, even worse, have negative psychological effects. Thus other means of therapies are needed to alleviate patient stress. Previous studies have indicated that histological tumour differentiation is a strong predictor to the venous or lymphatic permeation of cancer cells and invasion patterns of colon cancers and gastric cancers [10, 11]. This suggests that histological tumour differentiation [3, 6, 12] and lymph node metastasis [13–17] could be a good predictor when designing therapeutic strategies of the common-type pancreatic cancer. Therefore it is worthwhile to evaluate these potential biological properties and provide predictive information of cancer cell behaviour pre-operatively.

Inductive logic programming (ILP) is a useful method to deal with the problem of finding a set of hypotheses (rules) covering positive examples and at the same time excluding negative examples. It uses first-order logic as a uniform representation for examples and hypotheses. The ILP technique provides us a platform to generate several rules that indicate the relationship between the pancreatic cancer and the related factors. ILP provides an algorithm to learn hypotheses, expressed in logic, from a database by assuming the followings:

(a) background knowledge B in the form of a Prolog program;
(b) some language specification L describing the hypotheses;
(c) an optional set of constraints I on acceptable hypotheses;
(d) a finite set of examples E [18].

Here, E is the union of non-empty set of 'positive' examples $E+$ and a set of 'negative' examples $E-$. The aim of an ILP is to find a set of rules (H), in the form of a logic program, that cover all the positive examples without negative examples. ILP has distinct advantages than other data-mining techniques because it can facilitate the interaction between humans and computers by using background knowledge to narrow the search space and return human-comprehensible results, therefore taking advantage of both the computer's speed and the human's knowledge and skills.

Using ILP model, we are able to develop three rules for pancreatic cancer. The first rule demonstrates that large tumour size is a strong predictor of the presence of lymph nodal metastasis. The patients with the small tumour size could have a higher survival rate. The second hypothesis reveals that a patient with high levels of CA19-9 and carcinoembryonic antigen (CEA) are highly suggestive of malignancy. Furthermore, the abnormality of these two products can serve as vital biomarker. The final hypothesis indicates the high serum Elastase I could be a diagnostic clue to detect pancreatic cancer, and CEA in the serum is significantly higher in patients with pancreatic carcinoma. Thus, the measurements of CEA and Elastase I are very useful for the detection of pancreatic cancer. These three rules are worthwhile to provide information about the underlying property of pancreatic cancer. Therefore, it will greatly benefit the cancer patients for having better therapeutics.

2 Methods

In our study, 438 surgically resected and histologically confirmed [19, 20] common-type pancreatic cancer cases at the National Cancer Center Hospital are utilised for the analysis. With the approval of the National Cancer Center Institutional Review Board, we undertake the ILP technique to identify several rules related to the pancreatic cancer. Based on the pathological reports information, lymph node metastatic status ($N0$: negative nodal metastasis or $N+$: positive nodal metastasis) and tumour differentiation status are used as the basis of classification. We prepare two data sets based on the above classification criteria: Set Diff (tumour differentiation between poorly differentiated against others) and the Set $N1$ ($N+$ against $N0$). For Set Diff, poorly to moderately differentiated tumour samples are considered as positive samples (305), and well-differentiated tumour samples are taken as negative samples (133). As for as Set $N1$ is concerned, we define $N0$ as negative findings while set the others as positive, with a total of 353 positive samples. We retrieve laboratory data from the same cancer cases: CEA, CA19-9, Glucose, Elastase I, Serum Amylase, C-reactive protein (CRP), Serum Glucose (GLU), Fibrin degradation product (FDP), Fibrinogen (FIBG) and Antithrombin III (ATIII). We also retrieve data regarding age, sex, tumour location, tumour size (TS mm), number of lymphocytes (LymphNum) and lymphocyte ratio (Lymph-Cell). Fig. 1 summarises the pancreatic observations.

To identify the important features, we perform feature ranking using all available feature selection criteria, including 'Bhattacharyya', 't-test', 'ROC', 'entropy' and 'Wilcoxon'. These criteria assess the significance of every feature for separating two labelled groups. The Bhattacharyya criterion is based on the minimum attainable classification error [21]. The empirical receiver operating characteristic (ROC) curve and the random classifier slope are computed in [22]. Entropy computes the Kullback–Leibler distance or divergence [23], and Wilcoxon uses the Mann–Whitney test [24].

After feature ranking, we select the features that are highly ranked for the Set Diff: CA19-9, CEA, FIBG, Elastase I, TS, Location and GLU. For Set $N1$, the following features are consistently extracted among the top features: CA19-9,

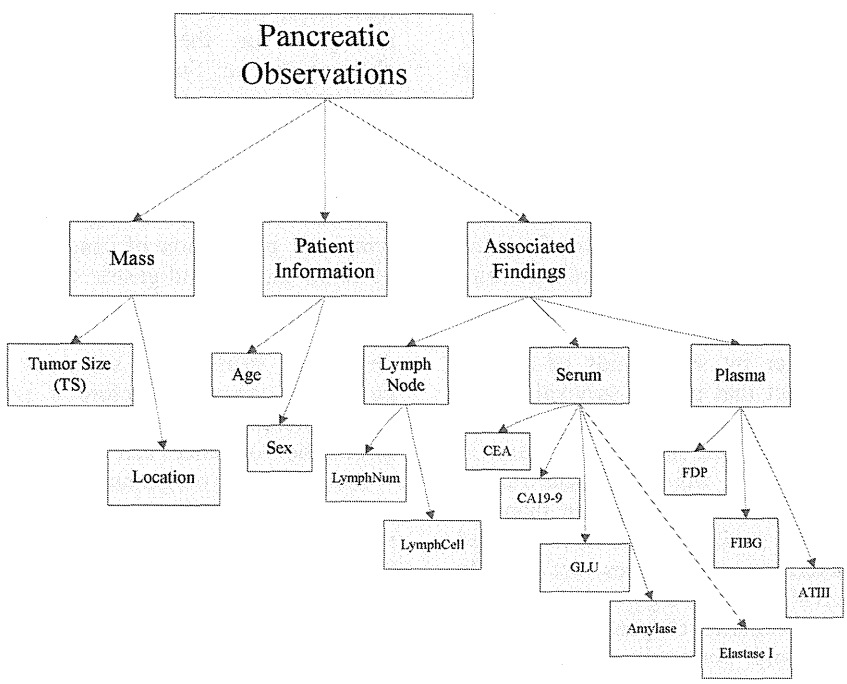

Fig. 1 *Pancreatic observation*

doi: 10.1049/iet-syb.2013.0044