

Serum tumor antigen REG4 as a diagnostic biomarker in pancreatic ductal adenocarcinoma

Reiko Takayama · Hidewaki Nakagawa · Akira Sawaki · Nobumasa Mizuno · Hiroki Kawai · Masahiro Tajika · Yasushi Yatabe · Keitaro Matsuo · Rie Uehara · Ken-ichiro Ono · Yusuke Nakamura · Kenji Yamao

Received: 25 November 2008 / Accepted: 30 June 2009 / Published online: 30 September 2009
© Springer 2009

Abstract

Background and Aims Serum biomarkers for the early detection of pancreatic cancer are not currently available. We evaluated the usefulness of a novel serum marker, REG4, in the diagnosis of pancreatic cancer, as compared to carbohydrate antigen (CA) 19-9.

Electronic supplementary material The online version of this article (doi:10.1007/s00535-009-0114-y) contains supplementary material, which is available to authorized users.

R. Takayama · A. Sawaki · N. Mizuno · K. Yamao (✉)
Department of Gastroenterology, Aichi Cancer Center Hospital,
1-1 Kanokoden, Chikusa-ku, Nagoya, Aichi 464-0021, Japan
e-mail: kyamao@aichi-cc.jp

H. Kawai · M. Tajika
Department of Endoscopy, Aichi Cancer Center Hospital,
Nagoya, Japan

Y. Yatabe
Department of Pathology and Molecular Diagnosis,
Aichi Cancer Center Hospital, Nagoya, Japan

K. Matsuo
Division of Epidemiology and Prevention,
Aichi Cancer Center Hospital, Nagoya, Japan

H. Nakagawa · Y. Nakamura
Laboratory of Molecular Medicine, Human Genome Center,
Institute of Medical Science, The University of Tokyo,
Tokyo, Japan

R. Uehara · K. Ono
Medical and Biological Laboratories Co., LTD, Nagoya, Japan

R. Takayama
Department of Gastroenterology, Matsusaka Central Hospital,
Matsusaka, Japan

Methods We collected pretherapeutic sera from 92 patients with pancreatic cancer, as well as sera from 28 patients with other pancreatic tumors, 11 patients with pancreatitis, and 69 healthy controls. Serum levels of REG4 were measured using a standard sandwich enzyme-linked immunosorbent assay (ELISA).

Results Compared with healthy controls, serum levels of REG4 were higher in pancreatic cancer patients ($P < 0.001$), and in patients with pancreatitis ($P < 0.001$). Receiver operating characteristic (ROC) analysis indicated that serum REG4 performed better than serum CA19-9 for distinguishing patients with pancreatic cancer from healthy controls [areas under the curve (AUC) for REG4 and CA19-9 were 0.922 and 0.884, respectively]. When we validated the study, the sensitivity of REG4 for pancreatic cancer was 94.9%, specificity was 64.0%, and accuracy was 77.5% for the REG4 cutoff value of 3.49 ng/ml. No correlation was seen between serum REG4 and CA19-9 levels, with the sensitivity, specificity, and accuracy of the combined markers reaching 100.0, 60.0, and 77.5%, respectively. No significant differences were seen among any stages of pancreatic cancer. In surgical specimens, immunohistochemical analysis found a correlation between serum REG4 levels and REG4 expression in pancreatic cancers.

Conclusions REG4 is expressed in pancreatic cancer, and serum levels of REG4 offer a useful indicator for distinguishing between patients with pancreatic cancer and healthy subjects. Serum REG4 has potential for use as a screening serum marker for pancreatic cancers, including early-stage cancers.

Keywords REG4 · Serum tumor antigen · Biomarker · Pancreatic cancer

Introduction

Pancreatic cancer is the fourth leading cause of cancer-related deaths in Western countries and shows the worst mortality among common malignancies, with a 5-year survival rate of only 5% [1, 2]. Estimates from 2007 predicted 37170 newly diagnosed cases of pancreatic cancer, and noted that a roughly equal number of deaths would be attributed to pancreatic cancer in the United States [3]. The majority of pancreatic cancer patients are diagnosed at an advanced stage, at which point effective therapies are no longer available. At present, surgical resection is the only possibility for a cure, but 80–90% of patients who undergo curative surgery suffer relapses and die from metastatic or disseminated disease [4]. Some approaches utilize a combination of surgery with chemotherapy based on gemcitabine or 5-fluorouracil (5-FU), with or without radiotherapy, and such modalities have been shown to improve the quality of life in these patients [5]. However, such treatments for advanced or relapsed pancreatic cancer generally have very limited effect with regard to improvement of long-term survival, since pancreatic cancers are extremely biologically aggressive and they are usually chemotherapy- and radiation-resistant [5]. The National Cancer Institute Pancreatic Cancer Progress Review Group has found that only about 4% of diagnosed patients live for more than 5 years [6]. However, in the early stages of pancreatic cancer, prognosis is relatively hopeful, with the 5-year survival of patients with stage I tumors less than 2 cm in size and no metastasis being seen to reach more than 50% [7]. One of the most realistic strategies for dealing with this lethal disease is, thus, screening for the early stages, to enable patients to undergo margin-negative surgical resections and thus achieve a potential for cure [8].

However, early-stage pancreatic cancer is extremely difficult to diagnose, comprising less than 10% of newly diagnosed pancreatic cancers. The reason for this is that patients with early-stage pancreatic cancer are usually asymptomatic, and optimal imaging or diagnosis of small pancreatic lesions requires specialized and invasive skills, despite the rapid progress that has been made in improving imaging techniques [8]. Many investigators and clinicians have therefore sought to find accurate and sensitive non-invasive serum markers of pancreatic cancer. In addition, screening methods for early pancreatic cancer involve examining high-risk populations who smoke, have alcohol consumption-associated chronic pancreatitis, have a recent onset of diabetes, and have a family history of pancreatic cancer [9].

Carbohydrate antigen (CA) 19-9 is currently the most widely used serum marker available. However, American Society of Clinical Oncology (ASCO) 2006 guidelines for the use of tumor markers do not recommend CA19-9 as a

screening test for pancreatic cancer. The reason for this is that CA19-9 is not specific for pancreatic cancer and is also elevated in many other gastrointestinal tumors and in inflammatory or benign conditions of the hepatobiliary system. Thus, CA19-9 may not be elevated in small malignant tumors of the pancreas [10]. Establishment of screening strategies through the development of novel serum markers that are more specific and more sensitive to pancreatic cancer is thus urgently needed.

To identify target molecules for diagnosis and therapy of pancreatic cancer, in earlier experiments, we generated precise genome-wide gene expression profiles of pancreatic cancer cells by combining our genome-wide cDNA microarrays with laser beam microdissection [11]. Among the numerous *trans*-activated genes found in pancreatic cancer cells, we focused on a recently discovered member of the regenerating islet-derived (REG) family as a tumor marker of pancreatic cancer, REG4 [12]. The REG family comprises secreted proteins that are speculated to play a role in tissue regeneration and inflammation in digestive organs [13–15]. Expressions of members of the REG family have been reported to be upregulated in several gastrointestinal cancers and to function as trophic or anti-apoptotic factors in cancers [15, 16]. Our previous study validated the overexpression of REG4 in pancreatic cancer cells through an immunohistochemical analysis that used anti-REG4 antibody. We were able to detect REG4 in a small set of pancreatic cancer sera when using an enzyme-linked immunosorbent assay (ELISA), suggesting potential for REG4 use as a serum marker for pancreatic cancers.

The previous study also established an ELISA system that was able to detect serum REG4 more sensitively. Subsequently, we were able to coordinate a large clinical study using this REG4 ELISA method to evaluate whether serum REG4 detection could be used as a diagnostic marker for pancreatic cancer [12].

Patients, materials, and methods

Clinical samples

We selected a population of 120 patients with pancreatic tumors, including ductal adenocarcinoma, adenosquamous carcinoma, anaplastic carcinoma, acinar cell adenocarcinoma, intraductal papillary mucinous neoplasm (IPMN), endocrine tumor, mucinous cystic neoplasm (MCN), serous cystic neoplasm (SCN), and solid pseudopapillary tumor (SPT), along with 11 patients with pancreatitis and 69 healthy controls. We categorized ductal adenocarcinoma, adenosquamous carcinoma, anaplastic carcinoma, invasive carcinoma derived from intraductal tumor, and intraductal papillary mucinous carcinoma (IPMC) as pancreatic

cancers. Sera were collected from 92 patients with pancreatic cancer [stage IA $n = 1$, IB $n = 1$, IIA $n = 4$, IIB $n = 6$, III $n = 22$, and IV $n = 58$; International Union Against Cancer (UICC) staging]. In addition, we collected sera from 16 patients with intraductal papillary mucinous adenoma (IPMA), 4 patients with endocrine tumor, 2 patients with acinar cell carcinoma, 2 patients with MCN, 3 patients with SCN, 1 patient with SPT, 11 patients with pancreatitis, and 69 healthy controls (Supplementary Data 1). Sera were collected from patients with pancreatic tumors between November 2004 and June 2006 for the purpose of evaluating candidate serum markers of pancreatic cancer. For the validation study, we collected sera from 39 patients with pancreatic cancer and 50 healthy controls between October 2007 and February 2008. Appropriate informed consent was obtained from all patients and healthy volunteers, and blood was collected from patients prior to any therapeutic procedures, including surgery, chemotherapy, and radiotherapy. Diagnoses of each pancreatic tumor were derived from histopathological resection, by fine-needle aspiration cytology, or by endoscopic findings (endoscopic retrograde cholangiopancreatography and/or endoscopic ultrasonography, along with clinical information). All sera were collected using standard procedures and stored at -80°C until analysis. All study protocols were approved by the Aichi Cancer Center Committee for Clinical Investigation.

ELISA

Serum levels of REG4 were measured by standard sandwich ELISA, as previously described [12]. Briefly, a 96-well immuno-module microplate (Nalgen Nunc International, Rochester, NY, USA) was precoated with monoclonal antibody (mAb) to REG4 (clone 21-1; MBL, Nagoya, Japan) by incubation overnight at 4°C , followed by blocking for 2 h at room temperature. Fivefold diluted sera were reacted to biotinylated anti-REG4 polyclonal (p) Ab for 15 min and added to an assay plate precoated with anti-REG4 mAb. After incubation for 2 h, the plates were washed five times to remove any unbound antibody-enzyme reagent; this was followed by the addition of 8000-fold diluted horseradish peroxidase (HRP)-labeled streptavidin (GE Healthcare, Piscataway, NJ, USA) with the reaction allowed to proceed for 1 h. After five washes, TMB substrate solution (Moss, Pasadena, MD, USA) was added to the wells and allowed to react for 15 min. The reaction was determined using a photometer at a wavelength of 450 nm and a reference wavelength of 620 nm. For the calibration curve of REG4 ELISA, we prepared recombinant REG4, which was used as the antigen to generate mAb to REG4, and this was diluted serially from 25 ng/ml to 176 pg/ml ($n = 8$) in a 96-well microplate.

REG4 ELISA was performed on this set. CA19-9 was also measured using a commercially available ELISA assay. Personnel blinded to the diagnosis of patients performed all analytical measurements.

Immunohistochemistry

Sections from paraffin-embedded blocks were deparaffinized and autoclaved for 15 min at 108°C in citrate buffer (pH 6.0). Endogenous peroxidase activity was quenched by incubation for 30 min in 0.33% hydrogen peroxide diluted in methanol. After incubation with fetal bovine serum for the purpose of blocking, sections were incubated with anti-REG4 rabbit pAb for 30 min at room temperature (MBL; 1:1000). After washing with phosphate-buffered saline (PBS), immuno-detection was performed using peroxidase-labeled anti-rabbit immunoglobulin (Envision kit; Dako Cytomation, Carpinteria, CA, USA). Finally, reactants were developed with 3, 3'-diaminobenzidine (Dako Cytomation) with the cells, then counterstained with hematoxylin. A single expert pathologist (Y. Y.) evaluated all of the immunostaining patterns and histopathology of the pancreatic tissues.

Statistics

To clarify the ability to distinguish controls and patient cases, REG4 and CA19-9 levels were evaluated using the Mann-Whitney U test. Pairwise comparisons between normal healthy controls and each pancreatic cancer stage group were also performed. In total, 14 statistical tests were applied for this evaluation. A two-way comparison of markers was made among normal healthy controls and patients with pancreatic cancers or other pancreatic tumors. Eight statistical tests were conducted. Values of $P < 0.05$ were considered statistically significant. To avoid false-positive results, we adjusted P values for multiple comparisons. As 22 statistical tests were applied, adjusted P values were calculated as the P value obtained $\times 22$. Receiver operating characteristic (ROC) curves were used to assess diagnostic properties. The threshold of each marker was defined to obtain maximum sensitivity and specificity. All statistical analyses were performed using SPSS II for Windows version 11.0.1J (SPSS, Chicago, IL, USA).

Results

Development and characterization of REG4 ELISA

We established an ELISA to detect serum REG4 by employing a mouse mAb to REG4 as the capture reagent and biotinylated rabbit polyclonal anti-REG4 antibody as

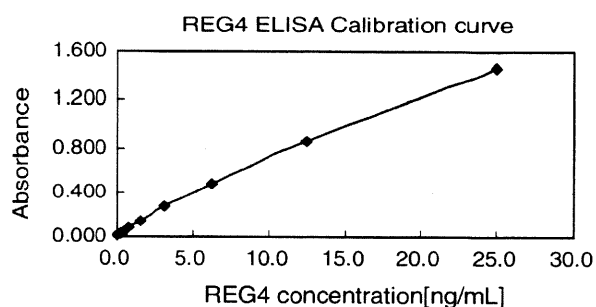


Fig. 1 REG4 enzyme-linked immunosorbent assay (ELISA) calibration curve. The X axis shows the concentration of the recombinant REG4 protein (ng/ml). For the Y axis, the absorbance used was 490 nm, with 630 nm used as the reference for the ELISA reaction. The lower limit of detection for the ELISA was 176 pg/ml, while saturation occurred for REG4 concentrations of more than 200 ng/ml. A linear range was determined to occur within a REG4 antigen range of 176 pg/ml to 25 ng/ml

Table 1 Serum REG4 and CA19-9 levels in each group of pancreatic diseases

Characteristics	REG4 (ng/ml)	CA19-9 (U/ml)
Invasive ductal carcinomas (<i>N</i> = 92)		
Median	5.00	212.63
Range	1.26–64.47	0.14–44835
Other pancreatic tumors (<i>N</i> = 28)		
Median	3.16	17.59
Range	1.93–11.41	0.00–187.4
Pancreatitis (<i>N</i> = 11)		
Median	5.89	8.46
Range	2.38–11.78	0.00–84.20
Others (pancreatic cyst and fatty deposition) (<i>N</i> = 5)		
Median	3.13	3.76
Range	1.94–4.09	0.00–87.40
Healthy controls (<i>N</i> = 69)		
Median	2.39	7.23
Range	1.30–4.20	0.00–35.40

CA carbohydrate antigen

the detection reagent. The lower limit of detection for the ELISA was 176 pg/ml, while saturation occurred at REG4 concentrations of more than 200 ng/ml. A linear range was determined to occur from 176 pg/ml to 25 ng/ml of REG4 antigen (Fig. 1). The intrassay (*n* = 8) coefficients of variation (CV) were less than 10% (range 2.32–9.91%).

Serum REG4 level in pancreatic diseases

Using the above-described solid ELISA, we analyzed serum levels of REG4 in a total of 205 individuals. Table 1 shows median serum REG4 values for pancreatic cancer, other pancreatic tumors, pancreatitis, other benign

pancreatic disease, and normal healthy controls. Significant differences were identified between serum REG4 levels in pancreatic cancer and healthy controls ($P < 0.001$; Mann–Whitney *U* test; Fig. 2a). We defined the cutoff value as the level of REG4 providing the highest accuracy (85.1%), which included levels of REG4 for patients with pancreatic cancer and those of healthy controls. We also performed a validation study with a cutoff REG4 value of 3.49 ng/ml, and the sensitivity of REG4 for pancreatic cancer was 94.9%, specificity was 64.0%, and accuracy was 77.5% (Supplementary Data 2).

Comparison of diagnostic accuracies of serum REG4 and CA19-9

Simultaneously, we analyzed serum levels of CA19-9 in the same population, using ELISA. Table 1 shows the median CA19-9 levels in each subject group. Although significant differences were seen between serum CA19-9 levels in pancreatic cancer patients and healthy controls ($P < 0.001$; Mann–Whitney *U* test; Fig. 2b), a wide range was noted for the pancreatic cancers. ROC analysis was performed to evaluate the diagnostic utility of REG4 and CA19-9 results, as these values were identified as independent predictors of pancreatic cancer. REG4 performed better than CA19-9 in distinguishing patients with pancreatic cancer from healthy controls, with the AUC values for REG4 and CA19-9 determined to be 0.922 and 0.884, respectively (Fig. 3).

Serum REG4 and CA19-9 levels for each stage of pancreatic cancers

We categorized the 92 pancreatic cancers according to clinical stage and compared serum levels of CA19-9 and REG4 in each stage (Table 2). While our study also showed that serum CA19-9 levels in patients with advanced pancreatic cancer (stages III, IV) were markedly elevated, serum CA19-9 levels in patients with relatively early stages of pancreatic cancer (stages I, II) were very low (Fig. 2c). The significant difference was not so apparent between serum CA19-9 levels in patients with stages I + II (*n* = 12) and healthy controls (median 36.86 vs. 7.23 U/ml; $P = 0.154$, Mann–Whitney *U* test; Fig. 2c).

Similar to the CA19-9 results, serum REG4 levels in patients with advanced pancreatic cancer (stages III, IV) were also significantly elevated compared to those in healthy controls ($P < 0.001$; Mann–Whitney *U* test; Fig. 2d). Of note was the significant difference between serum REG4 levels in patients with early-stage pancreatic cancer (stages I + II) and those in healthy controls (median 12.4 vs. 2.39 ng/ml; $P < 0.001$; Mann–Whitney *U* test;

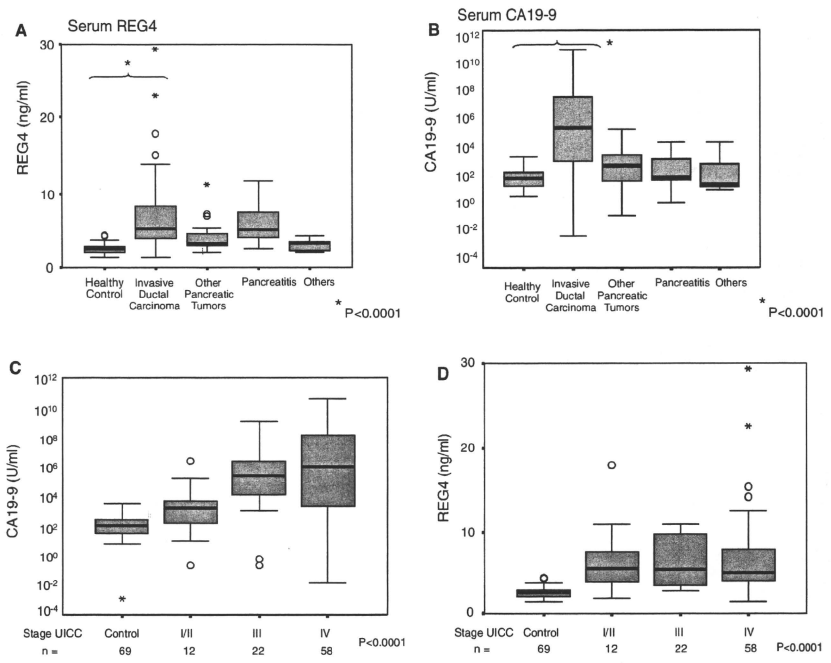


Fig. 2 a Serum REG4 levels in various pancreatic diseases. Serum REG4 was significantly elevated in patients with pancreatic cancer, as compared with healthy controls ($P < 0.001$; Mann–Whitney U test). Pancreatitis patients also showed elevated serum REG4 as compared with healthy controls ($P < 0.001$). b Serum carbohydrate antigen 19-9 (CA19-9) levels in various pancreatic diseases. Serum CA19-9 levels were significantly elevated in patients with pancreatic cancer, as compared with healthy controls ($P < 0.001$; Mann–Whitney U test). As some patients with advanced pancreatic cancer and other pancreatic tumors showed markedly high serum CA19-9 levels, a logarithmic function was used for the Y axis of this chart (\log_{10} CA19-9). c Patients with advanced pancreatic cancer [International Union

Against Cancer (UICC) stages III/IV; $n = 22 + 58$] showed higher levels of CA19-9 than healthy controls ($P < 0.001$; Mann–Whitney U test). However, no significant difference was apparent between healthy controls ($n = 69$) and patients with relatively early-stage pancreatic cancer (stages I/II; $n = 12$; $P = 0.154$). As the serum CA19-9 level was markedly high during stage IV pancreatic cancer, a logarithmic function was used for this chart. d Patients with advanced pancreatic cancer (stages III/IV; $n = 22 + 58$) showed higher REG4 levels than healthy controls ($P < 0.001$; Mann–Whitney U test). In addition, a significant difference ($P < 0.001$; Mann–Whitney U test) was seen between healthy controls ($n = 69$) and patients with relatively early-stage pancreatic cancer (stages I/II; $n = 12$)

Fig. 2d). In addition, two patients with stage I pancreatic cancer showed elevated REG4 levels in conjunction with normal CA19-9 serum levels.

Correlation between REG4 and CA19-9 and combined diagnostic ability

To provide additional information on how the serum markers behaved when used in combination in each group

of subjects, we created scatter plots of serum CA19-9 and REG4 levels (Fig. 4). The data showed no correlation between serum REG4 and CA19-9 levels, with a correlation coefficient (R^2) of 0.11. The results also indicated that serum REG4 could possibly behave as a serum marker for pancreatic cancer, unlike CA19-9. With the combined use of serum REG4 and CA19-9 measurements, sensitivity was 100%, with 60.0% specificity and 77.5% accuracy (Supplementary Data 2).

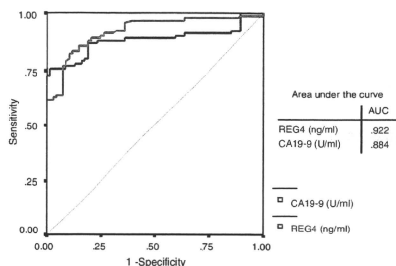


Fig. 3 Receiver operating characteristic (ROC) curve analyses for the use of REG4 (red line) and CA19-9 (blue line) as serum markers for pancreatic cancers. The X axis shows 1 – specificity, while the Y axis displays sensitivity for the detection of pancreatic cancer. The AUCs for REG4 and CA19-9 were 0.922 and 0.884, respectively. Serum REG4 was better than serum CA19-9 for distinguishing patients with pancreatic cancer from healthy controls

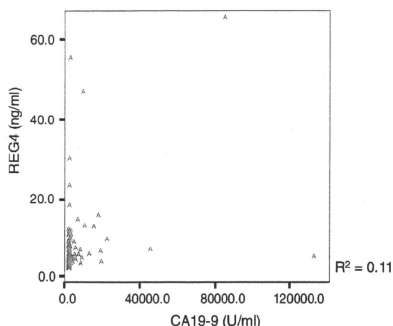


Fig. 4 Scatter plot for serum CA19-9 (X axis) and serum REG4 (Y axis). No correlation was seen between the serum REG4 level and the serum CA19-9 level (correlation coefficient, $R^2 = 0.11$). Serum REG4 could possibly behave as a serum marker for pancreatic cancer, different from CA19-9

Table 2 Serum REG4 and CA19-9 levels in each stage of pancreatic cancer

UICC stage	REG4 (ng/ml)	CA19-9 (U/ml)
I A/B (N = 2)		
Median	12.43	35.86
Range	7.0–17.9	23.6–48.1
II A/B (N = 10)		
Median	4.71	22.70
Range	1.70–11	0.50–588.3
III (N = 22)		
Median	5.22	212.63
Range	2.60–54.8	0.5–10159
IV (N = 58)		
Median	4.86	394.65
Range	1.30–64.5	0.1–44835
Total (N = 92)		
Median	5.00	212.63
Range	1.30–64.5	0.1–44835

UICC International Union against Cancer

Immunohistochemistry for REG4 expression

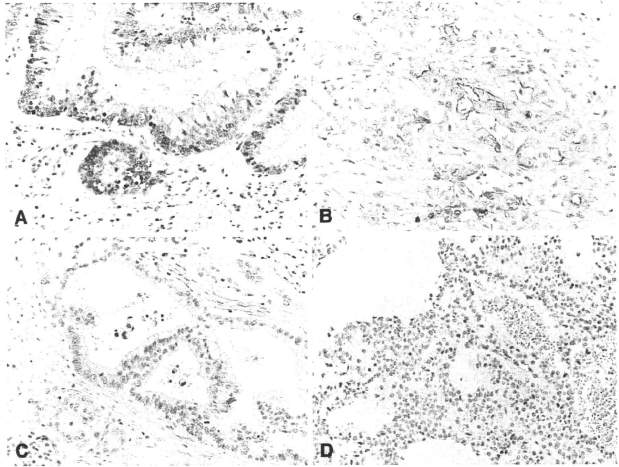
To confirm that the serum REG4 detected by our ELISA methodology was derived from tumor cells, we performed immunohistochemical analysis, using anti-REG4 pAb in 22 pancreatic tumor specimens (pancreatic cancers and other tumors). These specimens were surgically resected from individuals showing increases of more than 3.49 ng/ml ($n = 11$) or normal levels ($n = 11$) of serum REG4 prior to resection (Fig. 5a–d). Experienced pathologists

reviewed each histological preparation, and decided the intensity of REG4 expression. Summarizing the correlation between serum REG4 levels and REG4 expression in pancreatic tumors, 9 of the 11 cases in which the patients showed an increased level of serum REG4 also showed strong positive immunostaining of tumor cells, while only 2 of the 11 cases in which the patients had normal serum levels of REG4 exhibited positivity (Supplementary Data 3). A significant correlation was seen between serum REG4 level and REG4 expression in tumor cells ($P < 0.05$; χ^2 test), suggesting that the elevated serum REG4 levels in patients with pancreatic tumors could be derived from tumor cells.

Discussion

Our recent study demonstrated that approximately half of pancreatic cancer patients showed overexpression of REG4, determined using an ELISA system [12]. In addition, we detected a subset of this group showing elevated serum REG4 [11]. However, a few studies have reported that REG4 expression occurs mainly in gastrointestinal diseases, such as colorectal [17] and gastric cancers [18], and inflammatory bowel disease [19]. A large population-based study was required to verify the sensitivity and specificity of serum REG4 testing. Our preliminary study, using sera from dozens of patients with various types of cancers including pancreatic, gastric, and colorectal cancers, showed that patients with pancreatic and gastric cancer had the most significantly elevated levels of REG4 in sera. In Japan, gastric cancer is very common, and

Fig. 5 Immunohistochemical analysis for REG4 expression. **a** Invasive papillary mucinous carcinoma (stage IA) in a patient with serum REG4 level of 17.9 ng/ml exhibited strong positive staining for REG4 immunohistochemistry (IHC). **b** Well-differentiated ductal adenocarcinoma (stage IIB) in a patient with serum REG4 level of 10.95 ng/ml exhibited strong positive staining for REG4 IHC. **c** Well-differentiated ductal adenocarcinoma (stage IIA) in a patient with serum REG4 level of 1.73 ng/ml exhibited weak positive staining for REG4 IHC. **d** Solid pseudopapillary tumor (SPT) in a patient with serum REG4 level of 1.93 ng/ml exhibited no staining for REG4 IHC



endoscopic screening for gastric cancer is commonly performed; this screening can effectively detect early-stage gastric cancer without serum markers. Serum markers for gastric cancer would thus have less impact, and simultaneous examination of the upper gastrointestinal tract is usually performed by endoscopic examination for pancreatic diseases. The present study therefore focused only on pancreatic cancer, since no screening system is available for pancreatic cancer at present. Novel serum markers for pancreatic cancer are thus urgently required to identify ways to diagnose or screen for early-stage pancreatic cancer.

Although serum markers such as CA19-9 and carcinoembryonic antigen (CEA) are highly elevated in patients with advanced pancreatic cancer, these conventional markers are not elevated in early-stage or small pancreatic cancer, and thus are not useful for detecting such cancers. Serum REG4 is likely to be at least as effective as serum CA19-9 in distinguishing patients with invasive pancreatic cancer from healthy controls, and since serum REG4 behaves in a different manner from serum CA19-9, their use in combination might provide greater power to distinguish patients with invasive pancreatic cancer from healthy controls. Notably, the present study included two patients with early-stage pancreatic cancer (stage I) who showed elevated REG4 levels along with normal CA19-9 levels in sera. This suggests that serum REG4 might have some potential to help detect

early-stage pancreatic cancer. However, to clearly determine this, further studies analyzing a large number of patients with early-stage pancreatic cancer are required. With regard to pancreatitis, eight of eleven patients with pancreatitis in the present study showed elevated serum REG4 levels. In an examination of various normal adult organs, we confirmed that REG4 was expressed in pancreatic acinar cells, although the levels of REG4 expression in the acinar cells, as determined by immunohistochemical analysis, were much lower than those normally seen in cancer cells. Hence, in patients with pancreatitis, serum REG4 may be derived from destroyed acinar cells. As elevated serum levels of amylase or elastase have sometimes been observed in individuals with early-stage pancreatic cancer [20, 21], serum REG4 elevation in early-stage or small pancreatic cancers may reflect a localized pancreatitis around the cancer foci or tissue regeneration accompanied by cancer development, in addition to REG4 secretion from cancer cells. Recent studies have indicated the existence of a high-risk population for pancreatic cancer, such as patients with a strong family history of pancreatic cancer or patients with recent onset of diabetes. Pancreatic screening for such patients, using endoscopic ultrasonography and computed tomography, might therefore be beneficial [22, 23]. In addition, a simple and noninvasive serum examination, such as that for REG4, could be used in pancreatic surveillance for these high-risk

individuals, and could help to cure the pancreatic cancer more efficiently with early detection.

In conclusion, we have shown the promising feasibility of using REG4 as a serum tumor marker in helping to distinguish patients with pancreatic cancer from healthy controls. Also, we have just started additional validation studies in a multicenter test set of pancreatic cancer, other pancreatic tumors, and pancreatitis. These studies will, it is hoped, lead to the establishment of new methods of diagnosis for early-stage or small pancreatic cancers.

Acknowledgments We would like to thank Ms. Hitomi Uchida and Ms. Hiromi Kato for their technical assistance with the immunohistochemical analyses.

Conflict of interest statement There are no relevant conflicts of interest to disclose.

References

- DiMagno EP, Reber HA, Tempero MA. AGA technical review on the epidemiology, diagnosis, and treatment of pancreatic ductal adenocarcinoma. *Gastroenterology*. 1999;117:1464–84.
- Wray CJ, Ahmad SA, Matthews JB, Lowy AM. Surgery for pancreatic cancer: recent controversies and current practice. *Gastroenterology*. 2005;128:1626–41.
- Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. *CA Cancer J Clin*. 2007;57:43–66.
- Yeo CJ, Cameron JL, Lillemoe KD, Sitzmann JV, Hruban RH, Goodman SN, et al. Pancreaticoduodenectomy for cancer of the head of the pancreas: 201 patients. *Ann Surg*. 1995;221:721–31.
- Sener SF, Fremgen A, Menck HR, Winchester DP. Pancreatic cancer: a report of treatment and survival trends for 100, 313 patients diagnosed from 1985–1995, using the National Cancer Database. *J Am Coll Surg*. 1999;189:1–7.
- National Cancer Institute Pancreatic Cancer Progress Review Group. <http://prg.nci.nih.gov/pancreatic/finalreport.html> (2001).
- Egawa S, Takeda K, Fukuyama S, Motoi F, Sunamura M, Matsuno S. Clinicopathological aspects of small pancreatic cancer. *Pancreas*. 2004;28:235–40.
- Rosty C, Goggins M. Early detection of pancreatic carcinoma. *Hematol Oncol Clin North Am*. 2002;16:37–52.
- Hassan MM, Bondy ML, Wolff RA, Abbruzzese JL, Vauthey JN, Pisters PW, et al. Risk factors for pancreatic cancer: case-control study. *Am J Gastroenterol*. 2007;102:1–12.
- 2006 Update of ASCO recommendations for the use of tumor markers in gastrointestinal cancer. *J Oncol Pract* 2006;2:314–6.
- Nakamura T, Furukawa Y, Nakagawa H, Tsunoda T, Ohigashi H, Murata K, et al. Genome-wide cDNA microarray analysis of gene-expression profiles in pancreatic cancers using populations of tumor cells and normal ductal epithelial cells selected for purity by laser microdissection. *Oncogene*. 2004;23:2385–400.
- Takehara A, Eguchi H, Ohigashi H, Ishikawa O, Kasugai T, Hosokawa M, et al. Novel tumor marker REG4 detected in serum of patients with resectable pancreatic cancer and feasibility for antibody therapy targeting REG4. *Cancer Sci*. 2006;97:1191–7.
- Hartupe JC, Zhang H, Bonaldo MF, Soares MB, Dieckgraefe BK. Isolation and characterization of a cDNA encoding a novel member of the human regenerating protein family, Reg IV. *Biochim Acta*. 2001;1518:287–93.
- Watanabe T, Yonekura H, Terazono K, Yamamoto H, Okamoto H. Complete nucleotide sequence of human *reg* gene and its expression in normal and tumoral tissues. The *reg* protein, pancreatic stone protein, and pancreatic thread protein are one and the same product of the gene. *J Biol Chem*. 1990;265:7432–9.
- Unno M, Itoh T, Watanabe T, Miyashita H, Moriizumi S, Teraoka H, et al. Islet beta-cell regeneration and *reg* genes. *Adv Exp Med Biol*. 1992;321:61–6.
- Sekikawa T, Fukui H, Fujii S, Takeda J, Nanakin A, Hisatsune H, et al. REG I alpha protein may function as a tropic and/or anti-apoptotic factor in the development of gastric cancer. *Gastroenterology*. 2005;128:642–53.
- Violette S, Fester E, Pandrea-Vasile I, Mitchell V, Adida C, Dussaulx E, et al. Reg IV, a new member of the regenerating gene family, is overexpressed in colorectal carcinomas. *Int J Cancer*. 2003;103:185–93.
- Oue N, Hamai Y, Mitani Y, Matsumura S, Oshimo Y, Aung PP, et al. Gene expression profile of gastric carcinoma: identification of genes and tags potentially involved in invasion, metastasis, and carcinogenesis by serial analysis of gene expression. *Cancer Res*. 2004;64:2397–405.
- Kamarainen M, Heiskala K, Knuutila S, Heiskala M, Winqvist O, Andersson LC. RELP, a novel human REG-like protein with up-regulated expression in inflammatory and metaplastic gastrointestinal mucosa. *Am J Pathol* 2003;163:11–20.
- Ito T, Kimura T, Nawata H. Serum elastase 1 appears specific for cancer of the pancreatic head. *Am J Gastroenterol*. 1991;86:1778–83.
- Hayakawa T, Kondo T, Shibata T, Kitagawa M, Katada N, Kato K, et al. Prospective trial for early detection of pancreatic cancer by elevated serum immunoreactive elastase. *Gastroenterol Jpn*. 1990;25:727–31.
- Brentnall TA, Bronner MP, Byrd DR, Haggitt RC, Kimmey MB. Early diagnosis and treatment of pancreatic dysplasia in patients with a family history of pancreatic cancer. *Ann Surg*. 1995;221:721–31.
- Canto MI, Goggins M, Yeo CJ, Griffin C, Axilbund JE, Brune K, et al. Screening for pancreatic neoplasia in high risk individuals. *Clin Gastroenterol Hepatol*. 2004;2:606–21.

Clinical impact of radiotherapy for locally advanced pancreatic cancer

Akira Sawaki · Noriyuki Hoki · Satoko Ito · Kazuya Matsumoto · Nobumasa Mizuno · Kazuo Hara · Tadayuki Takagi · Yuji Kobayashi · Yugo Sawai · Hiroki Kawai · Masahiro Tajika · Tsuneya Nakamura · Kenji Yamao

Received: 16 February 2009 / Accepted: 29 July 2009 / Published online: 25 August 2009
© Springer 2009

Abstract

Background Although a randomized controlled trial for locally advanced pancreatic cancer (PC) has demonstrated a survival advantage for treatment with gemcitabine alone, chemoradiotherapy remains the treatment of choice for locally advanced disease in Japan. The aim of this study was to compare the survival benefits associated with gemcitabine and concurrent chemoradiotherapy in locally advanced unresectable PC.

Patients Seventy-seven patients with locally advanced unresectable PC were retrospectively enrolled from April 2001 to December 2006. All cases were histologically proven, and patients received gemcitabine chemotherapy ($n = 30$) or concurrent chemoradiotherapy (based on 5-fluorouracil, $n = 28$, or gemcitabine, $n = 19$, as a radiosensitizer) at Aichi Cancer Center Hospital.

Results Patients who received chemoradiotherapy had significantly better performance status than those who had chemotherapy. Tumor response was 0% for chemotherapy

and 13% chemoradiotherapy, but survival benefit was similar among patients in the chemotherapy group (overall response (OS) 12 months; progression-free survival (PFS), 3 months) and those in the chemoradiotherapy group (OS, 13 months; PFS, 5 months). Two-year survival was 21% for chemotherapy patients and 19% for chemoradiotherapy patients. Severe toxicities (Grade 3–4 National Cancer Institute-Common Toxicity Criteria, version 3.0) were significantly more frequent for chemoradiotherapy than for chemotherapy.

Conclusions Gemcitabine chemotherapy showed similar survival benefit compared to 5-fluorouracil- and gemcitabine-based chemoradiotherapy.

Keywords Locally advanced pancreatic cancer · Chemoradiotherapy · Chemotherapy

Introduction

Pancreatic cancer (PC) is a major unsolved health problem, accounting for approximately 20,000 deaths per year in Japan. Moreover, the prevalence of PC is increasing. Only a minority of PCs are resectable [1–3], and median survival is limited to between 11 and 23 months; 5-year survival is only approximately 20% even if surgical resection with or without adjuvant therapy is performed [2, 3]. Locally advanced disease is observed in 15–20% of all patients with PC, and is associated with a median survival of 6–10 months [4–6]. Locally advanced PC is defined as surgically unresectable because of extensive peripancreatic lymph node involvement, encasement or occlusion of the superior mesenteric vein or portal vein confluence, or direct involvement of the superior mesenteric artery, celiac axis, inferior vena cava, or aorta [7, 8]. Four randomized control

A. Sawaki (✉) · K. Matsumoto · N. Mizuno · K. Hara · T. Takagi · Y. Kobayashi · Y. Sawai · K. Yamao
Department of Gastroenterology, Aichi Cancer Center Hospital,
1-1 Kanokoden Chikusa-ku, Nagoya, Aichi 464-8681, Japan
e-mail: asawaki@aichi-cc.jp

N. Hoki
Department of Gastroenterology,
Bell Land General Hospital, Sakai, Japan

S. Ito
Department of Gastroenterology,
Shimane University School of Medicine, Izumo, Japan

H. Kawai · M. Tajika · T. Nakamura
Department of Endoscopy, Aichi Cancer Center Hospital,
Nagoya, Japan

trials [9–12] have compared the effectiveness of chemoradiation incorporating 5-fluorouracil (5-FU) with radiation alone or systemic chemotherapy. Three of these trials [9–11] showed an improved median survival of 10.1–10.6 months for radiotherapy plus 5-FU alone or triple therapy (streptozocin, mitomycin C and 5-FU: SMF) compared with 5.7–6.3 months for radiotherapy alone or systemic chemotherapy with SMF. Chemoradiotherapy has since been considered a standard therapy for locally advanced PC.

Burris et al. [13] documented a large randomized control trial in which weekly administration of gemcitabine was compared with 5-FU in the first-line treatment of unresectable PC. The efficacy of gemcitabine was indicated by significantly prolonged time to progression and to survival (1-year survival: 2–18%) demonstrating the existence of a subpopulation of PC patients who benefit from chemotherapy. Gemcitabine is widely accepted as the standard care for patients with unresectable PC. In 2008, Chauffert et al. [14] randomly divided 119 patients into either 59 external beam radiotherapy plus cisplatin and 5-FU followed by gemcitabine or 60 gemcitabine alone, and observed an improved median survival of 13 months for gemcitabine alone, compared with 8.6 months for chemoradiotherapy followed by gemcitabine. However, this finding remains contentious because the study enrolled fewer patients than planned and because median survival of the gemcitabine arm was longer than that indicated from historical data. If chemoradiotherapy conferred no survival benefit, gemcitabine alone could be a first-line treatment for locally advanced PC. We, therefore, conducted a retrospective analysis of data for patients with locally advanced PC treated with systemic chemotherapy or chemoradiotherapy, to evaluate the effect of the additional radiation.

Patients and methods

Endpoint

This retrospective study was designed to evaluate efficacy of chemoradiotherapy for patients with locally advanced PC. The primary endpoint was overall survival (OS); secondary endpoints were response rate, progression-free survival (PFS), and feasibility.

Patients

Consecutive patients with a histological diagnosis of pancreatic adenocarcinoma who were treated at the Aichi Cancer Center Hospital between April 2001 and November 2006 were retrospectively identified through medical

records. Endoscopic ultrasound-guided fine-needle aspiration and CT or MRI were used for diagnosis and staging, respectively. Additional eligibility criteria were as follows: (1) stage III or IV disease according to the UICC classification [15]; all involved lymph nodes within the radiation field if stage IV disease; (2) no history of other malignancy that could affect survival; (3) main organ function well preserved; (4) no previous chemotherapy or radiotherapy for PC. Finally, 77 patients with histologically proven, locally advanced, unresectable PC who were followed up until March 2008 constituted the study cohort. Although every effort was made to follow patients, patients were censored at the time of last contact if they were lost to follow-up. Measurable disease as defined by the Response Evaluation Criteria in Solid Tumors [16] (RECIST) was not mandatory. Written informed consent for treatment had been obtained from each patient prior to treatment.

Treatment regimens

Thirty patients who had received gemcitabine as initial chemotherapy were identified. Gemcitabine (1000 mg/m² as a 30 min infusion) was administered once weekly for 3 weeks followed by a 1-week rest period, until clinical progression or intolerable adverse event(s).

Forty-seven patients were treated with radiotherapy combined with 5-FU (28 patients) or gemcitabine (19 patients) as a chemosensitizer. The decision of whether to use 5-FU or gemcitabine was reached after discussion between patient and doctor. The 5-FU based chemoradiotherapy consisted of a total radiation dose of 50 Gy given in 25 daily fractions with a continuous intravenous infusion of 5-FU (375 mg/m²) daily, on the days that radiation therapy was administered. On the other hand, in gemcitabine-based chemoradiotherapy, weekly gemcitabine (250 mg/m²) was given instead of 5-FU. For all patients on chemoradiotherapy, after completing the treatment protocol, gemcitabine (1000 mg/m² as a 30 min infusion) was given until disease progression.

Efficacy and toxicity

Responses of measurable lesions to chemotherapy were evaluated using the RECIST guidelines every 8 weeks or earlier if there were indications of treatment failure due to toxicity. The outcome of complete response (CR) or partial response (PR) was confirmed by an independent radiologist. PFS was measured from the date of initial chemotherapy to date of progressive disease or death from any cause. Patients were censored if second-line treatment was initiated in the absence of progressive disease. OS was estimated from the date of initial treatment to the date of death or last follow-up visit.

Toxicity was evaluated using the National Cancer Institute-Common Toxicity Criteria [17] (version 3.0). Severe toxicities were defined as grade 3–4 (non-hematological) and grade 4 (hematological).

Statistical analysis

PFS and OS were calculated using the Kaplan–Meier method. PFS and OS of each treatment group were compared using the log-rank test. *P*-values for testing differences between proportions and response rate were calculated with chi-square tests for homogeneity or for trend, or with Fisher’s exact test.

All analyses were performed using SPSS version 12 (SPSS, Chicago, IL, USA) statistical software. Statistical results were considered significant when the *p*-value was <0.05. All reported *p*-values are two-sided.

Results

Patient characteristics

Patient characteristics did not differ significantly between the chemotherapy and chemoradiotherapy groups, with the exception of performance status (Table 1). PS was better in patients given chemoradiotherapy than those given chemotherapy (*p* = 0.002). Chemotherapy patients were relatively older than chemoradiotherapy patients (median age 64 vs. 57 years, *p* = 0.310). Forty-seven (61.0%) patients had a tumor in the pancreatic head and 24 (31.2%) underwent biliary drainage.

Tumor response, progression-free survival, and overall survival

None of the 30 chemotherapy patients responded (response rate 0%); 23 patients (76.7%) had progressive disease (Table 2). Of the 28 patients who received 5-FU-based chemoradiotherapy, 4 achieved a partial response, and of the 19 remaining patients who had gemcitabine-based chemoradiotherapy, 2 showed partial response. Overall response rate in the whole chemoradiotherapy group was 12.8%. Progressive disease was composed of radiological and clinical progression. 29 of 37 cases treated with chemoradiation were showed radiological progression and 12 of 23 with gemcitabine were diagnosed as progression by radiology. Table 3 revealed site(s) of progression according to chemoradiation or chemotherapy. There were no statistically significant differences between two treatments. Local control yield of radiation was not indicated.

Two-year and three-year survival was 21% and 0% for chemotherapy patients and 19% and 4% for

Table 1 Patient characteristics

	CRT (<i>n</i> = 47) [<i>n</i> (%)]	CT (<i>n</i> = 30) [<i>n</i> (%)]
Median age (range)	58 (31–79)	58 (31–79)
Gender		
Male	26 (55.3)	24 (80)
Female	21 (44.7)	6 (20)
PS (ECOG) at recurrence ^a		
0	29 (61.7)	8 (26.7)
1	18 (38.3)	22 (73.3)
Pathological stage		
Stage III	43 (91.5)	28 (93.3)
Stage IV	4 (8.5)	2 (6.7)
Location		
Head	30 (63.8)	17 (56.7)
Body	16 (34)	12 (40)
Tail	1 (2.2)	1 (3.3)
Size (median cm)	4.0	3.5
CEA (median ng/ml)	4.1	2.5
CA19-9 (median U/ml)	660.0	362.1
Biliary drainage		
Yes	14 (29.8)	10 (33.3)
No	33 (70.2)	20 (66.7)

^a Statistical significant difference in PS (*p* = 0.002)

PS performance status, ECOG Eastern Cooperative Oncology Group, CEA carcinoembryonic antigen, CA19-9 cancer antigen 19-9

Table 2 Response

	<i>n</i>	CR	PR	SD	PD	NE	RR (%)	DCR (%)
CRT	47	0	6	4	37	0	12.8	21.3
CT	30	0	0	7	23	0	0	23.3

CRT chemoradiotherapy, CT chemotherapy, CR complete response, PR partial response, SD stable disease, PD progressive disease, NE not evaluable, RR response rate, DCR disease control ratio (CR + PR + SD/all)

Table 3 Progression pattern

Site(s) of progression	CRT <i>n</i> = 29	CT <i>n</i> = 12	<i>p</i> value
Local	13	5	0.40
Distant	16	6	0.18
Both	0	1	NE

CRT chemoradiotherapy, CT chemotherapy, NE not evaluable

chemoradiotherapy, respectively. Although response rate differed significantly between chemotherapy and chemoradiotherapy (*p* = 0.041), it was similar between gemcitabine and 5-FU. Median PFS was 3.0 months in the chemotherapy group and 5.0 months in the chemoradiotherapy group (*p* = 0.333, Fig. 1). After a median follow-up of

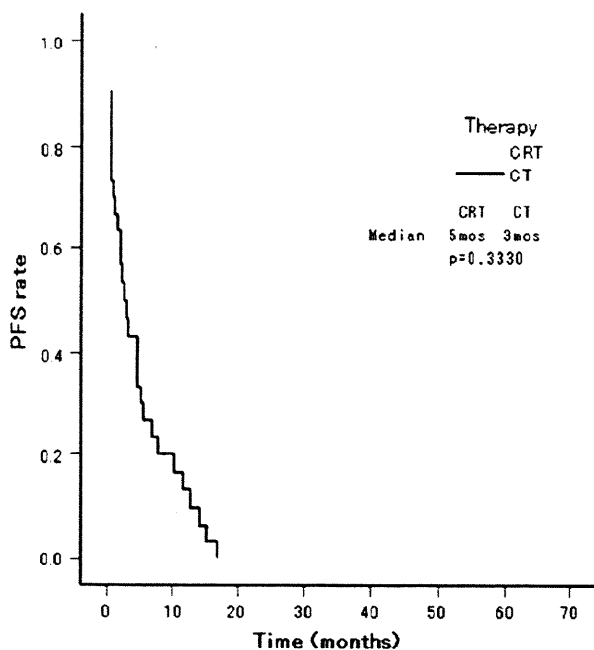


Fig. 1 Median progression-free survival in the chemotherapy (CT) and chemoradiotherapy (CRT) groups was 3 months and 5 months, respectively

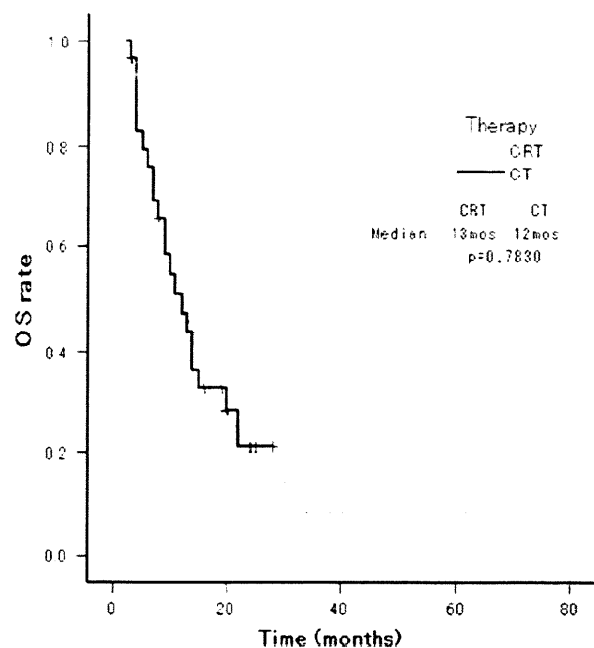


Fig. 2 Overall survival was 12 months in the chemotherapy (CT) group and 13 months in the chemoradiotherapy group (CRT)

11 months, OS was 12 months in the chemotherapy group and 13 months in the chemoradiotherapy group. Although OS was longer in the chemoradiotherapy group, this difference was not statistically significant ($p = 0.7830$, Fig. 2).

Toxicity

Grade 3–4 toxicities are detailed in Table 4. Most of these non-hematological adverse effects were digestive (nausea, vomiting, diarrhea, anorexia, and hemorrhage), and they were significantly more frequent in the chemoradiotherapy patients than in the chemotherapy patients (95.7% vs. 20.0%; $p < 0.001$). More serious hematological toxicities were reported in chemoradiotherapy (10.6% of patients had neutropenia and 12.8% had thrombocytopenia) than in chemotherapy patients (3.3% had neutropenia). There was statistical difference between chemoradiotherapy and chemotherapy in overall hematological toxicities ($p = 0.030$). In chemosensitizer, there was no difference in toxicities between 5-FU and gemcitabine treatment groups.

Discussion

A phase III trial of advanced PC [13] showed a definite benefit of gemcitabine for unresectable PC that included locally advanced as well as metastatic disease, but it raised the issue of efficacy of radiotherapy in locally advanced

PC. In 1969, Moertel et al. [9] reported the first randomized control trial to evaluate the efficacy of chemoradiation therapy for the treatment of locally advanced PC. The OS in the chemoradiotherapy group was significantly better than that in the radiotherapy group. Since this and other trials [11, 12] demonstrated the efficacy of radiotherapy, chemoradiotherapy has been performed in our hospital in the treatment of locally advanced PC. Radiotherapy (60 Gy radiation) has been combined with 5-FU, administered as an intravenous continuous infusion (375 mg/day).

Ben-Josef et al. [18] reported that median survival with chemoradiotherapy is around 10–12 months and that 1-year survival is approximately 40–50%. The present study found very similar median OS (13 months) and 1-year survival (54%) for chemoradiotherapy patients. Median and 1-year survival for chemotherapy were 12 months and 47%, respectively, and there were no significant differences in these measures between the chemoradiotherapy and chemotherapy groups. In the current study, chemoradiotherapy was performed with 5-FU or gemcitabine (250 mg/m^2) as a chemosensitizer. Although the chemosensitizer was selected on the basis of discussion between patient and doctor, gemcitabine tended to be chosen for younger patients with better general status. Nonetheless, median survival did not differ significantly between the two chemosensitizers (5-FU, 14 months; gemcitabine, 12 months). A previous study showed that toxicity related to gemcitabine chemoradiotherapy tended

Table 4 Severe toxicity

Toxicity	CRT (<i>n</i> = 47) [<i>n</i> (%)]	CT (<i>n</i> = 30) [<i>n</i> (%)]
Non-hematological toxicities		
Nausea and vomiting	19 (40.4)	2 (6.7)
Diarrhea	5 (10.6)	0 (0)
Anorexia	18 (38.3)	3 (10.0)
Exanthema	0 (0)	1 (3.3)
Hemorrhage	3 (6.4)	0 (0)
Stomatitis	2 (4.3)	0 (0)
Overall ^a	45 (95.7)	6 (20.0)
Hematological toxicities		
Leucopenia	5 (10.6)	1 (3.3)
Neutropenia	1 (2.1)	0 (0)
Thrombocytopenia	6 (12.8)	0 (0)
Overall ^a	12 (25.5)	1 (3.3)

^a Statistical significant differences in non-hematological ($p < 0.001$) and hematological ($p = 0.03$)

to be more severe than that related to 5-FU chemotherapy [19]. However, we did not find significant differences in toxicity between those given chemoradiotherapy and those given chemotherapy. This may be because patients were not randomly assigned to groups and those with better general status were given gemcitabine based chemoradiation (see Tables 1, 4).

Radiation is superior to chemotherapy in terms of local control, so why did the present trial fail to show the efficacy of radiation? First, PC cells have low sensitivity to radiation [20]. Second, it is possible that the diagnosis of locally advanced PC was not always accurate; CT and other diagnostic modalities cannot detect micro-metastasis. We have encountered patients with early relapse who show multiple liver metastases at first evaluation CT just after chemoradiotherapy. Some of these patients might have had small liver metastases at the start of chemoradiotherapy; in the present study they could have been diagnosed with locally advanced PC and given chemoradiotherapy. On the other hand, patients who survive a long time and do not develop metastasis or local relapse until more than 1 year after the start of treatment are more likely to represent those with 'true' locally advanced PC without distant metastasis. This type of disease would be well controlled by radiation. Inclusion of 'false' locally advanced disease may have accounted for the lack of differences in OS and progression pattern between two treatments, a difference between chemotherapy and chemoradiotherapy. A study limited to true locally advanced disease might show the effectiveness of chemoradiotherapy. Three-year survival of chemoradiotherapy and chemotherapy were 4.3% (2/47) and 0% (0/30), respectively.

Two longer survivors of 47 given chemoradiotherapy might benefit from radiotherapy.

In addition, medical cost is also important issue to evaluate two treatments. We calculated the medical cost from the start of treatment to day 56. Mean cost of chemoradiation and gemcitabine chemotherapy was about 9800 dollars and 5600 dollars, respectively. Since there are no differences between chemoradiation and chemotherapy, gemcitabine chemotherapy showed the advantage of gemcitabine chemotherapy in the aspect of the cost-effectiveness.

Both chemotherapy and chemoradiotherapy were feasible, but in terms of toxicity, chemoradiotherapy was associated with more frequent toxicities; especially neutropenia and gastrointestinal toxicity. Taking into consideration the fact that patients with better PS were given chemoradiotherapy, toxicity is likely to be much greater in chemoradiotherapy than in chemotherapy. Hence, to further explore innovative approaches, less toxic forms of chemoradiotherapy are needed.

In conclusion, there were no significant differences in survival benefit between gemcitabine chemotherapy and chemoradiotherapy; radiotherapy had no clinical impact for patients with locally advanced PC. Gemcitabine monotherapy was not associated with severe adverse events and therefore appears to be indicated for every locally advanced PC. Considering the efficacy and safety of the two forms of treatment, gemcitabine monotherapy was considered the optimal treatment for locally advanced PC.

Acknowledgments This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, Culture and Technology of Japan and by a Grant-in Aid for Cancer Research from the Ministry of Health, Labour and Welfare of Japan.

References

1. Heinemann V. Present and future treatment of pancreatic cancer. *Semin Oncol.* 2002;29:23–31.
2. Goggins M. Molecular markers of early pancreatic cancer. *J Clin Oncol.* 2005;23:4524–31.
3. Chua YJ, Cunningham D. Adjuvant treatment for resectable pancreatic cancer. *J Clin Oncol.* 2005;23:4532–7.
4. Heinemann V. Gemcitabine in the treatment of advanced pancreatic cancer a comparison analysis of randomized trials. *Semin Oncol.* 2002;29:9–16.
5. Schneider G, Siveke JT, Eckel F, Schmid RM. Pancreatic cancer; basic and clinical aspects. *Gastroenterology.* 2005;128:1606–25.
6. Abbruzzese JL. Past and present treatment of pancreatic adenocarcinoma: chemotherapy as a standard treatment modality. *Semin Oncol.* 2002;29:2–8.
7. Willent CG, Czito BG, Bendell JC, Ryan DP. Locally advanced pancreatic cancer. *J Clin Oncol.* 2005;23:4538–44.
8. Lockhart AC, Rothenberg ML, Berlin JD. Treatment for pancreatic cancer; current therapy and continued progress. *Gastroenterology.* 2005;128:1642–54.
9. Moertel CG, Childs DS Jr, Reitemeier RJ, Colby MY Jr, Holbrook MA. Combined 5-fluorouracil and supervoltage

- radiation therapy of locally unresectable gastrointestinal cancer. *Lancet*. 1969;2:865–7.
10. Moertel CG, Frytak S, Hahn RG, O'Connell MJ, Reitemeier RJ, Rubin J, et al. Therapy of locally unresectable pancreatic carcinoma a randomized comparison of high dose (6000 rads) radiation alone, moderate dose radiation (4000 rads + 5-fluorouracil), and high dose radiation + 5-fluorouracil. *Cancer*. 1981;48:1705–10.
 11. Gastrointestinal Tumor Study Group. Treatment of locally unresectable carcinoma of the pancreas: comparison of combined-modality therapy (chemotherapy plus radiotherapy) to chemotherapy alone. *J Natl Cancer Inst*. 1988;80:751–5.
 12. Klaassen DJ, MacIntyre JM, Catton GE, Engstrom PF, Moertel CG. Treatment of locally unresectable cancer of the stomach and pancreas: a randomized comparison of 5-fluorouracil alone with radiation plus concurrent and maintenance 5-fluorouracil-an Eastern Cooperative Oncology Group study. *J Clin Oncol*. 1985;3:373–8.
 13. Burris HA 3rd, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, et al. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol*. 1997;15:2403–13.
 14. Chauffert B, Mornex F, Bonnetain F, Rougier P, Mariette C, Bouché O, et al. Phase III trial comparing intensive induction chemoradiotherapy (60 Gy, infusional 5-FU and intermittent cisplatin) followed by maintenance gemcitabine with gemcitabine alone for locally advanced unresectable pancreatic cancer. Definitive results of the 2000-01 FFCD/SFRO study. *Ann Oncol*. 2008;19:1592–9.
 15. Sobin LH, Wittekind C, editors. UICC-TNM classification of malignant tumours, 6th ed. New York: Wiley-Liss; 2002.
 16. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst*. 2000;92:205–16.
 17. National Cancer Institute Common Toxicity Criteria. Version 3.0, 9 August 2006. <<http://ctep.cancer.gov/forms/CTCAEv3.pdf>>.
 18. Ben-Josef E, Lawrence TS. Chemoradiotherapy for unresectable pancreatic cancer. *Int J Clin Oncol*. 2008;13:121–6.
 19. Okusaka T, Ito Y, Ueno H, Ikeda M, Takezako Y, Morizane C, et al. Phase II study of radiotherapy combined with gemcitabine for locally advanced pancreatic cancer. *Br J Cancer*. 2004;91:673–7.
 20. Asanuma K, Moriai R, Yajima T, Yagihashi A, Yamada M, Kobayashi D, et al. Survivin as a radioresistance factor in pancreatic cancer. *Jpn J Cancer Res*. 2000;91:1204–9.

Association of prostate stem cell antigen gene polymorphisms with the risk of stomach cancer in Japanese

Keitaro Matsuo^{1,2*}, Kazuo Tajima¹, Takeshi Suzuki¹, Takakazu Kawase¹, Miki Watanabe¹, Kohei Shitara³, Kazunari Misawa⁴, Seiji Ito⁴, Akira Sawaki⁵, Kei Muro³, Tsuneya Nakamura⁶, Kenji Yamao⁵, Yoshitaka Yamamura⁴, Nobuyuki Hamajima⁷, Akio Hiraki⁸ and Hideo Tanaka^{1,6}

¹Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan

²Department of Epidemiology, Nagoya University Graduate School of Medicine, Nagoya, Japan

³Department of Medical Oncology, Aichi Cancer Center Central Hospital, Nagoya, Japan

⁴Department of Gastroenterological Surgery, Aichi Cancer Center Central Hospital, Nagoya, Japan

⁵Department of Gastroenterology, Aichi Cancer Center Central Hospital, Nagoya, Japan

⁶Department of Endoscopy, Aichi Cancer Center Central Hospital, Nagoya, Japan

⁷Department of Preventive Medicine/Medical Decision Making, Nagoya University Graduate School of Medicine, Nagoya, Japan

⁸Health Center, Okayama University, Okayama, Japan

A recent whole-genome association study identified a strong association between polymorphisms in the prostate stem cell antigen (PSCA) gene and stomach cancer risk. In this case-control study, we aimed to validate this association, and further to explore environmental factors possibly interacting with PSCA polymorphisms in 708 incident stomach cancer cases and 708 age–sex matched controls. The association between PSCA polymorphisms and *Helicobacter pylori* infection was also examined. We found that rs2294008 and rs2976392, which were strongly linked to each other ($D' = 1.00$), were significantly associated with stomach cancer risk. Per allele odds ratio for rs2994008 was 1.40 (95% confidence interval: 1.19–1.65; $p = 3.7 \times 10^{-5}$). We found significant interaction with a family history of stomach cancer in first-degree relatives (p -heterogeneity = 0.009). Similar to originally reported association, we found significant heterogeneity between diffuse and intestinal type (p -heterogeneity = 0.007). No association was seen between PSCA polymorphisms and *H. pylori* infection. In conclusion, PSCA polymorphisms are associated with stomach cancer risk in Japanese. A possible interaction with family history warrants further evaluation.
© 2009 UICC

Key words: stomach cancer; prostate stem cell antigen; polymorphism; case-control study

Although the age-standardized incidence of stomach in Japan is decreasing, the stomach remains one of the most common sites of cancer.¹ Worldwide, the health burden of stomach cancer in terms of incidence and mortality is similarly still high.² Although *Helicobacter pylori* infection is a well-established causative agent,³ epidemiologic studies exploring other risk/protective factors are still ongoing, including genetic factors.

A recent genome-wide association study (GWAS) conducted in Japanese and Korean populations reported a strong association between prostate stem cell antigen (PSCA) gene polymorphisms and the risk of stomach cancer. Findings showed that one of the polymorphisms examined, rs2294008 in the first exon, may be the locus responsible for changes in the transcriptional activity of an upstream fragment of PSCA.⁴ Despite the importance of this finding, the independence of this putative effect of PSCA loci from *H. pylori* infection in stomach carcinogenesis remains to be clarified. This study also did not investigate potential interactions with confounders.

Here, we conducted a case-control study with 3 goals: First, to validate the association between these PSCA polymorphisms and stomach cancer risk; Second, to explore the interaction between PSCA loci and confounders; and Third, to examine the association between the PSCA loci and *H. pylori* infection.

Material and methods

Study population

The present subjects were aged 20–79 years, and were enrolled between January 2001 and November 2005, in the framework of

the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC). Details of the HERPACC have been described elsewhere.^{5,6} In brief, the first version of the HERPACC (HERPACC-I) was initiated at Aichi Cancer Center Hospital, Nagoya, Japan, in 1988, with information on lifestyle factors collected from all first-visit outpatients, including cancer and non-cancer patients. The second version (HERPACC-II) was launched in 2001, and asked all first-visit outpatients to provide 7 ml of blood as well as information on lifestyle factors. Patients were asked about their lifestyle when healthy or before the current symptoms developed. Information from the HERPACC-II questionnaire was systematically collected and checked by trained interviewers. Complete responses were obtained from 96.7% of 29,538 eligible subjects, of whom 50.7% donated a blood sample. Questionnaire data were loaded into the HERPACC database and periodically linked with the hospital cancer registry system to update information on cancer incidence. All participants gave written informed consent, and the study was approved by the Ethics Committee of Aichi Cancer Center.

Cases and controls

A total of 708 patients who provided completed questionnaires and donated blood samples within the framework of HERPACC-II and were newly diagnosed with stomach cancer were deemed potential cases. Among cases, 71.6% were available for histologic information (274 cases: Intestinal type: 304 cases: Diffuse type and 130 cases: unknown).

Control subjects were randomly selected from first-visit outpatients who visited our hospital in the same period and enrolled in HERPACC-II. A total of 9,497 individuals who completed the questionnaire and donated blood samples and were confirmed to have no cancer according to the cancer registry and medical records were deemed potential controls. We excluded 642 patients with a history of cancer, leaving 8,855 controls eligible for analysis. We used non-cancer patients at our hospital as controls, given the likelihood that our cases arose within this population base. Eventually, 706 controls were individually matched for age (± 3 years) and sex to cases with a 1:1 case-control ratio. On examination at our hospital, 29% of controls had no abnormal

Grant sponsors: Ministry of Education, Science, Sports, Culture and Technology of Japan; Ministry of Health, Labour and Welfare of Japan.

*Correspondence to: Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan. Fax: +81-52-763-5233. E-mail: t-suzuki@aichi-cc.jp

Received 11 December 2008; Accepted after revision 31 March 2009

DOI 10.1002/ijc.24519

Published online 22 April 2009 in Wiley InterScience (www.interscience.wiley.com).

finding, 32% had digestive diseases (e.g., gastritis and gastric or colon polyp), 11% had respiratory diseases (e.g., non-specific chest shadow and benign tumors), 2% had benign breast diseases (e.g., fibroadenoma), 4% had gynecologic diseases (e.g., cervix dysplasia), 8% had head and neck diseases (e.g., benign tumors), and 14% had miscellaneous other conditions (e.g., skin and orthopedic disorders).⁷ Our previous study demonstrated that the general lifestyles of non-cancer subjects in HERPACC were accordant with those of the general population in the same area,⁸ warranting the external validity of the results of HERPACC-based studies. All subjects in the present study were Japanese living in and around Aichi Prefecture, Central Japan.

Genotyping of *PSCA* polymorphisms

DNA of each subject was extracted from the buffy coat fraction using BioRobot EZ1 and an EZ1 DNA Blood 350 ml Kit (Qiagen, Tokyo, Japan) or DNA Blood mini kit (Qiagen). Genotyping for the 2 loci in the *PSCA* gene (dbSNP ID: rs2294008, rs2976392 and rs2976391) was based on TaqMan assays by Applied Biosystems (Foster City, CA). In our laboratory, the quality of genotyping was routinely assessed statistically using the Hardy-Weinberg test. When allelic distributions for controls departed from the Hardy-Weinberg frequency, genotyping was assessed using direct sequencing.

Helicobacter assessment

Among 708 controls, 644 controls were examined for plasma IgG levels for *H. pylori* using a commercially available direct enzyme-linked immunosorbent assay (ELISA) kit ("E Plate 'Eiken' *H. Pylori* Antibody" from Eiken Kagaku, Tokyo, Japan). This ELISA kit was developed in Japan using the antigen extracted from the domestic strain in Japan and is commonly used in medical studies.^{9,10} A positive status for *H. pylori* infection was defined as an *H. pylori* IgG antibody level greater than 10 U/ml in serum. Serum pepsinogens (PG) were measured by chemiluminescence enzyme immunoassay (CLEIA). Severe gastric mucosal atrophy was defined as those with PG I \leq 30 ng/ml and PG I/PG II \leq 2.

Assessment of smoking exposures

Cumulative smoking dose was evaluated as pack-years, the product of the number of packs consumed per day and years of smoking. Smoking habits were entered under the 4 categories of never, former and current smoker of <40 and ≥ 40 pack-years. Former drinkers or smokers were defined as those who had quit drinking or smoking at least 1 year before the survey, respectively.

Statistical analysis

To assess the strength of the associations between *PSCA* polymorphisms and risk of stomach cancer, odd ratios (ORs) with 95% confidence intervals (CIs) were estimated using conditional logistic models adjusted for potential confounders. Potential confounders considered in the multivariate analyses were age, sex, smoking habit (never smokers, former smokers, current smokers of <40 , or ≥ 40 pack-years), and family history of stomach cancer in a first-degree relative (yes or no). Differences in categorized demographic variables between the cases and controls were tested by the chi-squared test. Accordance with the Hardy-Weinberg, equilibrium was checked for controls using the chi-squared test and used to assess any discrepancies between genotype and allele frequencies. Under the conditions of this study of 708 cases and 708 matched controls, and assuming a risk allele frequency 0.66 (according to rs2294008 in HapMap database for Japanese in Tokyo) with a per allele OR = 1.62⁴ and α -error of 0.001, statistical power was more than 0.99. To explore possible gene-environment interactions, we conducted stratified unconditional logistic regression analyses according to the adjusted factors. To avoid the dropping of subjects from models by stratification, a conditional

TABLE 1—CHARACTERISTICS OF CASES AND CONTROLS

Variable	Cases		Controls		<i>p</i> -value
Total	708		708		
Sex					1.00
Male	531	75.0%	531	75.0%	
Female	177	25.0%	177	25.0%	
Age (years)					0.446
<40	34	4.8%	35	4.9%	
40–49	72	10.2%	69	9.7%	
50–59	249	35.2%	222	31.4%	
60–69	216	30.5%	247	34.9%	
70–	137	19.4%	135	19.1%	
Mean age (SD)	59.4 (10.4)		59.8 (10.4)		
Smoking status ¹					<0.001
Never	242	34.2%	317	44.8%	
Moderate	84	11.9%	99	14.0%	
Heavy	375	53.0%	287	40.5%	
Unknown	7	1.0%	5	0.7%	
Family history of stomach cancer in the first degree relatives					0.032
No	646	91.2%	667	94.2%	
Yes	62	8.8%	41	5.8%	

¹Moderate and heavy smoker was defined as ever-smoker with pack-years less than 20 and more or equal to 20, respectively.

logistic model was not applied. We used the Mantel-Haenzel test to assess the homogeneity of association for *PSCA* by stratifying factors. The association between *PSCA* and *H. pylori* infection among controls was assessed by unconditional logistic regression for age, sex and smoking habit. We defined *p*-values less than 0.05 as statistically significant for the homogeneity test and *H. pylori* analysis.

All analyses were performed using STATA version 10 (Stata Corp., College Station, TX). Power calculations for sample sizes in gene-environment interactions were performed using QUANTO.¹¹

Results

Demographic features of cases and controls are shown in Table I. Age and sex were appropriately matched. Heavy smokers were more frequent among cases than controls. The proportion of 20 pack-years or more current smokers was significantly higher among cases than controls. Those with a family history of stomach cancer in a first-degree relative were more common among cases.

Table II shows genotype distributions for rs2294008, rs2976392 and rs2976391. Genotype frequencies for all polymorphisms were in accordance with the Hardy-Weinberg law in controls: rs2294008 ($p = 0.64$), rs2976392 ($p = 0.64$) and rs2976391 ($p = 0.36$). Allele frequencies in each locus were consistent with those in the HapMap database. rs2294008 and rs2976392 showed linkage disequilibrium (LD) ($D' = 1.00$ and $R^2 = 0.99$) while, rs2976391 did not show LD with the other two. rs2294008 showed a statistically significant association in all models. Table III shows stratified analysis according to sex, smoking, family history of stomach cancer and histologic subtypes. A significant interaction was seen for a family history of stomach cancer (p -homogeneity = 0.009), with those with a history showing a higher per-allele OR (2.98: 1.47–6.02) than those without (1.34: 1.14–1.59). Similar to the original report,⁴ we found significant impact of rs2294008 in diffuse type.

Among 642 controls (492 males and 150 females; mean age 60.8 years) examined for *H. pylori*, prevalence was 64.2% (95% confidence interval: 60.3–67.9). No significant association was seen between any *PSCA* polymorphism and *H. pylori*. The per allele OR adjusted for age, sex and smoking habit for rs2294008 was 0.97 (95% CI: 0.76–1.24, $p = 0.817$), indicating a lack of

TABLE II - GENOTYPES DISTRIBUTION OF PSCA POLYMORPHISMS AND THEIR ODDS RATIOS FOR STOMACH CANCER RISK

PSCA locus and genotype	Parallele model				Dominant model ¹				Recessive model ¹								
	Model 1 ¹		Model 2 ²		OR		p value		OR		p value		OR		p value		
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	
rs2294008: risk allele (A) frequency in controls subjects = 0.626																	
AA																	
GA																	
Case/control	49/97	329/338	330/273	1.40	1.19-1.65	1.35	1.14-1.59	3.9*10 ⁻⁴	2.07	1.45-2.95	6.4*10 ⁻⁵	1.37	1.11-1.68	0.003			
rs2976392: risk allele (A) frequency in controls subjects = 0.626																	
AA																	
GA																	
Case/control	48/96	328/337	331/274	1.40	1.19-1.65	1.36	1.15-1.60	2.9*10 ⁻⁴	2.09	1.46-2.99	5.7*10 ⁻⁵	1.36	1.10-1.67	0.004			
rs2976391: risk allele (C) frequency in 708 controls subjects = 0.804																	
AA																	
AC																	
CC																	
Case/control	13/31	206/215	488/461	1.25	1.03-1.52	1.21	0.99-1.48	0.061	2.50	1.28-4.88	0.007	1.19	0.95-1.49	0.126			

¹Crude conditional logistic regression model. ²Adjusted for smoking (non, moderate, heavy, unknown), and family history of stomach cancer in conditional logistic regression model.

association between PSCA polymorphism and *H. pylori* infection among this Japanese population. This association was not changed if we excluded subjects with sever gastric atrophy. Further, no associations for the other polymorphisms were seen.

Discussion

In this study, which had sufficient statistical power, we found a significant association between the PSCA polymorphisms rs2294008 and rs2976392 and stomach cancer risk. This association was consistent regardless of age, sex and smoking habit. Consistent with the former report, we found significant association with diffuse type gastric cancer. Interaction with a family history of stomach cancer in first-degree relatives was observed. The lack of association between these PSCA polymorphisms and *H. pylori* infection strengthen the independent impact of PSCA polymorphisms on stomach cancer risk.

To date, the background biological mechanism of PSCA and its transcript in stomach cancer has not been clarified. Sakamoto *et al.* suggested a role in signal transduction via a glycosylphosphatidylinositol anchor domain in PSCA, especially specific to diffuse type gastric cancer,¹² while several other reports have suggested an involvement in cell growth regulation in various systems.¹³⁻¹⁵ In an *in vitro* evaluation, HSC57 cells stably expressing PSCA grew more slowly than not expressing PSCA,⁴ supporting a potential role for PSCA in signal transduction. On the basis of their suppression of PSCA expression in epithelial cells in several tissues, including stomach, Sakamoto *et al.* also suggested a tumor suppressor-like function in certain types of cancer.⁴ Our present study does not clarify the significance of rs2294008 and rs2976392. rs2994008 is non-synonymous polymorphism in which the first methionine is changed to threonine, possibly leading to a difference between each allele. It was suggested that rs2294008 might modulate transcriptional activity of the upstream region of PSCA.⁴

We found that PSCA polymorphisms had a higher impact in those with a family history of stomach cancer than in those without. To our knowledge, this is the first report of this association. Several explanations can be considered. First, this phenomenon may simply reflect dominant nature of this polymorphism. Second, the prevalence of unknown loci in the PSCA gene or neighboring regions, which confer additional functional significance to the present subject loci and links with them, is high among those with a family history of stomach cancer. These loci might be difficult to detect in GWAS owing to their lower prevalence in those with a family history. Given this small ratio (8.8% in cases and 5.8% in controls), a third explanation may be chance. Further examination of this question is warranted.

As with other hospital-based case-control studies, our controls may have differed from the general population. However, the equivalence of genotype distributions for the PSCA polymorphism between our controls and those in the HapMap database for Japanese indicates a lack of bias in the selection of controls, and the strong association even after adjustment for potential confounders therefore confirms the solidness of the association. We did not assess the *H. pylori* infection status of cases, but rather assessed *H. pylori* status and PSCA polymorphisms. This is because the comparison of plasma/serum measurement between cases and controls in a case-control study is not necessarily an appropriate way to assess a causal relation. Given several findings that advanced gastric atrophy induces the elimination of *H. pylori* from the gastric mucosa, measurement of *H. pylori* does not necessarily reflect current/former infection status.¹⁶ To our knowledge, this is the first study to report a lack of association between PSCA polymorphisms and *H. pylori* infection.

In conclusion, our case-control study confirms a strong association between the PSCA polymorphisms rs2294008 and rs297639s and stomach cancer risk in the Japanese population. This association was independent of age, sex, smoking and drinking habits and

TABLE III – STRATIFIED ANALYSIS ACCORDING TO POTENTIAL CONFOUNDING FACTORS FOR PSCA RS2294008 GENOTYPE

Exposure	rs2294008 gene						<i>p</i> -homogeneity ²
	TT	CT	CC	Allele model ¹			
	Cases/controls	Cases/controls	Cases/controls	OR	95% CI	<i>p</i> -value	
Sex							
Male	39/68	247/259	244/204	1.35	1.12–1.64	0.002	0.30
Female	2008.9.28	81/78	87/70	1.62	1.17–2.25	0.003	
Smoking status ³							
Never	15/45	116/150	111/122	1.43	1.10–1.86	0.008	0.73
Moderate	3/15	43/46	38/38	1.54	0.96–2.47	0.07	
Heavy	31/36	169/139	175/112	1.31	1.03–1.67	0.026	
Unknown	0/1	2009.1.3	2009.6.1				
Family history of stomach cancer in the first degree relatives							
No	48/90	304/317	294/260	1.34	1.14–1.59	0.001	0.009
Yes	383.58	25/21	36/13	2.98	1.47–6.02	0.002	
Histology ⁴							
Diffuse	23/38	142/129	109/107	1.88	1.46–2.44	<0.001	0.007
Intestinal	15/44	127/148	162/112	1.15	0.89–1.50	0.27	

¹Odds ratios were adjusted for age and sex in unconditional logistic regression models. ²Mantel-Haenzel homogeneity test. ³Moderate and heavy smoker was defined as ever-smoker with pack-years less than 20 and more or equal to 20, respectively. ⁴130 cases were excluded from analysis because of lack of histology information. Corresponding 130 controls were also excluded.

family history of stomach cancer. The lack of association between these PSCA polymorphisms and *H. pylori* warrants the independence of their effect in stomach carcinogenesis. Further studies examining the biological mechanism behind this association are warranted.

Acknowledgements

The authors are grateful to many doctors, nurses and technical and administration staff of Aichi Cancer Center Hospital for the daily administration of the HERPACC study.

References

- Marugame T, Matsuda T, Kamo K, Katanoda K, Ajiki W, Sobue T. Cancer incidence and incidence rates in Japan in 2001 based on the data from 10 population-based cancer registries. *Jpn J Clin Oncol* 2007;37:884–91.
- WHO. The global burden of disease: 2004 Update. Switzerland: WHO, 2008.
- IARC. Schistosomes, liver flukes and *Helicobacter pylori*. Monographs on the evaluation of the carcinogenic risks to humans, vol. 61. Lyon: IARC Publishing, 1994:177–241.
- Sakamoto H, Yoshimura K, Saeki N, Katai H, Shimoda T, Matsuno Y, Saito D, Sugimura H, Tanioka F, Kato S, Matsukura N, Matsuda N, et al. Genetic variation in PSCA is associated with susceptibility to diffuse-type gastric cancer. *Nat Genet* 2008;40:730–40.
- Tajima K, Hirose K, Inoue M, Takezaki T, Hamajima N, Kuroishi T. A model of practical cancer prevention for out-patients visiting a hospital: the Hospital-Based Epidemiologic Research Program at Aichi Cancer Center (HERPACC). *Asian Pac J Cancer Prev* 2000;1:35–47.
- Hamajima N, Matsuo K, Saito T, Hirose K, Inoue M, Takezaki T, Kuroishi T, Tajima K. Gene-environment interactions and polymorphism Studies of Cancer Risk in the Hospital-based Epidemiologic Research Program at Aichi Cancer Center II (HERPACC-II). *Asian Pac J Cancer Prev* 2001;2:99–107.
- Hamajima N, Hirose K, Inoue M, Takezaki T, Kuroishi T, Tajima K. Age-specific risk factors of breast cancer estimated by a case-control study. *J Epidemiol* 1995;5:99–105.
- Inoue M, Tajima K, Hirose K, Hamajima N, Takezaki T, Kuroishi T, Tominaga S. Epidemiological features of first-visit outpatients in Japan: comparison with general population and variation by sex, age, and season. *J Clin Epidemiol* 1997;50:69–77.
- Sasazuki S, Inoue M, Iwasaki M, Otani T, Yamamoto S, Ikeda S, Hanaoka T, Tsugane S. Effect of *Helicobacter pylori* infection combined with CagA and pepsinogen status on gastric cancer development among Japanese men and women: a nested case-control study. *Cancer Epidemiol Biomarkers Prev* 2006;15:1341–7.
- Fukuda S, Shimoyama T, Umegaki N, Mikami T, Nakano H, Munkata A. Effect of *Helicobacter pylori* eradication in the treatment of Japanese patients with chronic idiopathic urticaria. *J Gastroenterol* 2004;39:827–30.
- Gauderman W, Morrison J. QUANTO documentation. (Technical report no. 157). Los Angeles, CA: Department of Preventive Medicine, University of Southern California, 2001.
- Reiter RE, Gu Z, Watabe T, Thomas G, Szigeti K, Davis E, Wahl M, Nisitani S, Yamashiro J, Le Beau MM, Loda M, Witte ON. Prostate stem cell antigen: a cell surface marker overexpressed in prostate cancer. *Proc Natl Acad Sci USA* 1998;95:1735–40.
- Saffran DC, Raitano AB, Hubert RS, Witte ON, Reiter RE, Jakobovits A. Anti-PSCA mAbs inhibit tumor growth and metastasis formation and prolong the survival of mice bearing human prostate cancer xenografts. *Proc Natl Acad Sci USA* 2001;98:2658–63.
- Gu Z, Yamashiro J, Kono E, Reiter RE. Anti-prostate stem cell antigen monoclonal antibody 1G8 induces cell death in vitro and inhibits tumor growth in vivo via a Fc-independent mechanism. *Cancer Res* 2005;65:9495–500.
- Tran CP, Lin C, Yamashiro J, Reiter RE. Prostate stem cell antigen is a marker of late intermediate prostate epithelial cells. *Mol Cancer Res* 2002;1:113–21.
- Ohata H, Kitauchi S, Yoshimura N, Mugitani K, Iwane M, Nakamura H, Yoshikawa A, Yanaoka K, Arii K, Tamai H, Shimizu Y, Takeshita T, et al. Progression of chronic atrophic gastritis associated with *Helicobacter pylori* infection increases risk of gastric cancer. *Int J Cancer* 2004;109:138–43.

Histological diagnosis of autoimmune pancreatitis using EUS-guided trucut biopsy: a comparison study with EUS-FNA

Nobumasa Mizuno · Vikram Bhatia · Waki Hosoda · Akira Sawaki · Noriyuki Hoki · Kazuo Hara · Tadayuki Takagi · Shigeru B. H. Ko · Yasushi Yatabe · Hidemi Goto · Kenji Yamao

Received: 2 September 2008 / Accepted: 27 February 2009 / Published online: 12 May 2009
© Springer 2009

Abstract

Purpose The aim of this study was to evaluate the feasibility and safety of endoscopic ultrasonography (EUS)-guided trucut biopsy (TCB) for diagnosis of autoimmune pancreatitis (AIP).

Methods Fourteen patients with suspected AIP based on imaging studies underwent both EUS-guided fine-needle aspiration (FNA) and EUS-TCB for diagnosis of AIP and exclusion of pancreatic cancer (PC). According to the revised Japanese clinical diagnostic criteria, AIP was diagnosed in eight while the remaining six patients had pancreatitis of other etiologies. Pathologically, AIP was defined as lymphoplasmacytic sclerosing pancreatitis (LPSP), and sub-divided into two types: definite LPSP (d-LPSP) showing fulspectrum of LPSP and probable LPSP (p-LPSP) without obliterative phlebitis or abundant (>10 cells/hpf) IgG4-positive plasmacytes infiltration.

Results PC was excluded in all patients. EUS-FNA resulted in three of eight patients with AIP were reported as p-LPSP, one was reported as normal, and 4 were inconclusive. One of six with non-autoimmune pancreatitis was diagnosed as p-LPSP on EUS-FNA, one as idiopathic chronic pancreatitis (ICP) and four were inconclusive. By using EUS-TCB, all AIP patients were diagnosed as LPSP (4 d-LPSP and 4 p-LPSP). Of the six patients with non-autoimmune pancreatitis, three were diagnosed as LPSP (1 d-LPSP and 2 p-LPSP) and three showed ICP on TCB. No complications were identified in any patient with either EUS-FNA or TCB.

Conclusion EUS-TCB is a safe and accurate procedure for obtaining a histological diagnosis in patients with suspected AIP. EUS-TCB can serve as a rescue technique in cases of AIP lacking typical findings.

Keywords AIP · LPSP · EUS-TCB · Pancreatic cancer

N. Mizuno (✉) · A. Sawaki · N. Hoki · K. Hara · T. Takagi · K. Yamao
Department of Gastroenterology, Aichi Cancer Center Hospital,
1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan
e-mail: nobumasa@aichi-cc.jp

V. Bhatia
Department of Medical Hepatology,
Institute of Liver and Biliary Sciences (ILBS),
New Delhi, India

W. Hosoda · Y. Yatabe
Department of Pathology and Molecular Diagnostics,
Aichi Cancer Center Hospital, 1-1 Kanokoden,
Chikusa-ku, Nagoya 464-8681, Japan

S. B. H. Ko · H. Goto
Department of Gastroenterology,
Nagoya University Graduate School of Medicine,
Nagoya, Japan

Introduction

Autoimmune pancreatitis (AIP) is now being increasingly diagnosed based on its unique clinical features, radiological images and serological findings [1–3]. Histopathological findings of AIP have typically been described in resected specimens since most cases of AIP are initially misdiagnosed as pancreatic cancer (PC), and obtaining adequate pancreatic tissue using non-surgical approaches is difficult [4–7]. Endoscopic ultrasonography (EUS)-guided fine-needle aspiration (FNA) is now widely accepted as a safe and effective modality for obtaining pancreatic tissue samples [8]. The diagnostic accuracy of FNA for PC is reported to be between 60% and 90%, but conclusive diagnosis of AIP is often difficult due to the small size of specimens obtained by

FNA [7, 9]. In view of this limitation, large-caliber cutting biopsy (trucut biopsy [TCB]) needles have been developed to acquire samples with preserved tissue architecture, thus allowing histological examination [10].

To date, several diagnostic criteria for AIP have been proposed from many countries such as Japan [11], Korea [12], United States (Mayo Clinic) [13], and countries of the European Union. Asian diagnostic criteria for AIP based on Japanese and Korean consensus were also proposed recently [14]. Among these, only the Mayo Clinic criteria (HISORt criteria) allow a conclusive diagnosis of AIP based on pancreatic histology without any radiological features or serological testing, when specific features of lymphoplasmacytic sclerosing pancreatitis (LPSP) are found on histology [13]. Although the HISORt criteria require a “core” biopsy for the diagnosis of LPSP, whether EUS-TCB is effective for providing an adequate histological core of the pancreas is unclear [15].

In May 2004, EUS-TCB of the pancreas was introduced at our hospital to obtain core pancreatic tissue from patients with suspected AIP. To date there is no study comparing EUS-FNA and EUS-TCB for diagnosis of AIP. Thus, the aim of this study was to evaluate the feasibility and safety of EUS-guided TCB (EUS-TCB) for the diagnosis of AIP, comparing it with the conventional EUS-FNA.

Patients and methods

This study was a retrospective case review of all patients who underwent both EUS-FNA and EUS-TCB for diagnosis of AIP and exclusion of PC. Between January 1997 and February 2008, we evaluated 36 patients in whom AIP was suspected because of pancreatic enlargement and narrowing of the main pancreatic duct (MPD) on computed tomographic (CT) imaging, magnetic resonance imaging (MRI), and/or endoscopic retrograde cholangiopancreatography (ERCP). After EUS-TCB was introduced at the Aichi Cancer Center, 14 of the above-mentioned patients underwent a pancreatic TCB to differentiate AIP from PC. All of the patients were non-drinkers with no family history of pancreatitis. Fourteen patients with PC who underwent both EUS-FNA and TCB were included in this study as control subjects.

Informed consent was obtained from all patients before the procedure. Collection of data for this study was approved by our Institutional Review Board. EUS-FNA of the pancreas was done with a disposable 22-gauge needle (EZ-Shot™, Olympus, Tokyo, Japan) advanced through a 2.8-mm channel linear echoendoscope (GF-UCT240, Olympus). A part of FNA sample was placed onto a glass slide and fixed in absolute alcohol solution for staining. Rest of FNA sample was fixed in formalin and embedded in

paraffin. When the on-site cytologic examination was negative for malignancy, then, a core biopsy specimen was obtained by EUS-TCB using a disposable 19-gauge trucut needle (QuickCore™, Wilson-Cook, Winston-Salem, NC) [16]. All tissue samples obtained by TCB were routinely fixed in formalin and embedded in paraffin. Deparaffinized sections 4- μ m thick were stained with hematoxylin and eosin. For immunohistochemical staining, a monoclonal anti-human immunoglobulin (IgG)4 antibody (Binding Site, Birmingham, UK) was used with standard immunohistochemical techniques. The extent of IgG4-positive plasma cells were scored as none, mild, moderate and marked according to the number of immunohistochemically identified positive staining plasma cells per high-power field (hpf) in each specimen. Tissues with less than 5 positive cells/hpf were scored as none, 5–10 cells/hpf were scored as mild, 11–30 cells/hpf scored as moderate, and tissues with >30 positive cells/hpf were scored as marked [17].

In this study, patients who met both criterion 1 and 2 of the revised clinical diagnostic criteria of AIP 2006 (revised Japanese criteria) were diagnosed with AIP. The following are the criteria: (1) typical pancreatic imaging features, (2) typical laboratory abnormalities and (3) histopathological examinations. First, we compared histopathological findings obtained by EUS-FNA and EUS-TCB with the clinical features. All tissue slides were reviewed by the same pathologist (W.H.), who was blinded to the clinical information. The histology of AIP, termed LPSP is characterized histologically by a dense lymphoplasmacytic infiltrate centered around the pancreatic ducts and ductules, accompanied by obliterative phlebitis, acinar atrophy and interstitial fibrosis (storiform fibrosis) [4, 18, 19]. LPSP was divided into two types: (1) definite LPSP, showing the full spectrum of LPSP changes with obliterative phlebitis (Fig. 1), and (2) probable LPSP when obliterative phlebitis was absent or abundant (>10 cells/hpf) IgG4 positive plasmacytes infiltration. Chronic pancreatitis with the presence of granulocyte epithelial lesion (GEL) was defined as idiopathic duct-centric chronic pancreatitis (IDCP) [4, 7]. When features of chronic pancreatitis were found pathologically, but findings of LPSP or IDCP were absent, it was defined as idiopathic chronic pancreatitis (ICP, Fig. 2). Second, we assessed the diagnostic usefulness of EUS-TCB in diagnosis of AIP comparing EUS-FNA, imaging examinations, laboratory findings and the revised Japanese criteria. Third, we evaluated the usefulness of EUS-FNA and TCB for differentiating between focal pancreatitis and PC.

Statistical analysis

The diagnostic performance of EUS-FNA and TCB were compared with the chi-squared test (using JMP version