the bulk mass of total protein in serum. Proteins of interest as potential biomarkers for malignant or non-malignant diseases (e.g. prostate-specific antigen, various interleukins and cytokines) are usually present in plasma/serum only at ng/mL to pg/mL levels. Because the dynamic range of MS is limited, it is difficult to detect these biomarker candidates among highly abundant proteins. We used an Ig-Y immunoaffinity column to increase the concentration of serum proteins with lower abundance. This procedure removed 12 highly abundant proteins with satisfactorily high reproducibility.[25] However, only proteins with relatively high abundance such as apolipoprotein A-IV and complement component C4A (Table 1), which have been implicated in human malignancies other than uterine endometrial cancer,[26,27] were identified, indicating that our LC-MS is still not looking deeply enough into candidate biomarker proteins with low abundance. It will be necessary to develop a more comprehensive prefractionation method for plasma/serum samples to exploit the full capacity of our method.

Although high-quality MS/MS spectra were obtained from nearly all peptides (data not shown), MS/MS data for only 11 peptides matched the amino acid sequences of proteins deposited in the database with high confidence ($P < 0.05$). The Human Proteome Organization (HUPO) recently completed the first large-scale collaborative study to characterize the human serum and plasma proteomes. Although 9504 proteins identified with one or more peptides, and 3020 proteins identified with two or more peptides, only 889 proteins were identified with a confidence level of at least 95%. The high rate of false identification may be attributable not only to novel exons in alternatively spliced variants of known proteins or previously nonannotated gene sequences,[28] but also to post-translational modification of proteins. Plasma/serum biomarker candidates have been reported to be invariably proteins modified with aberrant glycosylation, cleavage, or dimerization.[29–31] A low frequency of completely matched peptide sequences in the database is inevitable at this point. However, the reproducible quantification of marker peptides by MS could be directly applicable to clinical use without the need for actual protein identification.

We have demonstrated that large-scale quantitative proteomic comparison is readily possible using this new version of 2DICAL. 2DICAL is a quantitative proteome platform characterized by its simplicity and throughput and is now applicable to any kinds of large-scale biomarker discovery studies.

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Supporting information

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

Fig. S1. Reproducibility of 2-Dimensional Image Converted Analysis of Liquid chromatography and mass spectrometry (2DICAL). (a–c) Two-dimensional plots indicating the high reproducibility of mass spectrometry (MS) intensity (in log scale) between corresponding >150 000 peaks of a representative sample. The sample was run three times (Runs 1, 2, and 3). The average correlation coefficient (CC) values were 0.99 between Runs 1 and 2 (a), 0.96 between Runs 1 and 3 (b), and 0.97 between Runs 2 and 3 (c). (d) The median intensity of the entire MS peaks (>150 000 peaks) of triplicates was compared between two different days one week apart. The average CC value was 0.96.

Figs. S2–5. High-speed tandem mass spectrometry (MS/MS) analysis. Labeled MS/MS spectra and peak lists of ID 17519, ID 37148, ID 28122, and ID 14166, which matched the sequences of apolipoprotein A-IV, complement component C4A, complement component C3, and inter-α-trypsin inhibitor family heavy chain-related protein (IHRP), respectively.

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CASE REPORT OF INTEREST

p53 gene mutation and p53 protein overexpression in a patient with simultaneous double cancer of the gallbladder and bile duct associated with pancreaticobiliary maljunction

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Abstract Pancreaticobiliary maljunction (PBM) is associated with the occurrence of biliary cancer due to pancreatobiliary reflux. We present a case of simultaneous double cancer of the gallbladder and bile duct. A 77-year-old woman who had jaundice, intra- and extra-hepatic biliary ductal dilatation and a space-occupying lesion in the gallbladder and lower bile duct underwent pancreatoduodenectomy. The gallbladder cancer showed papillary carcinoma without mutation of the K-ras gene and with p53 non-sense mutation of CCA (*Pro*) to CA (*Stop*) on codon 301 in exon 8. The bile duct cancer revealed a well-differentiated adenocarcinoma without mutation of the K-ras gene and with p53 miss-sense mutation of GTG (*Val*) to GAG (*Glu*) on codon 272 in exon 8. There were no mutations of either the K-ras or p53 gene in non-cancerous epithelia. In contrast, only the mucosa of the common channel had p53 protein accumulation and high cell proliferation activity. Therefore, the genetic pathway might be the same in both the gallbladder and bile duct cancer, and a high potential for carcinogenesis might be present in the epithelium of the common channel in patients with PBM.

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Introduction

Pancreaticobiliary maljunction (PBM) is a congenital anomaly defined as a union of the pancreatic and biliary ducts outside of the duodenal wall [1–3]. PBM develops because of an arrest in the migration of the choledochopancreatic junction into the duodenal wall before the eighth week of gestation and shows a long common channel with the absence of a septum between the bile duct and the pancreatic duct [1–3]. The high risk of PBM for biliary tract cancer has been reported [2]. Pancreatic juice refluxes into the biliary tract and pools in the gallbladder and bile duct, resulting in activation of pancreatic enzymes, and is associated with the pathogenesis of biliary cancer. Therefore, the atypical epithelium frequently found in the common channel is the most important site of pathogenesis of cancer of the papilla of Vater [4]. Genetic analysis shows multiple genetic mutations, among which K-ras gene activation and the p53 tumor suppressor gene inactivation in the mucosa of the gallbladder and bile duct are recognized as the most important keys for carcinogenesis in PBM [5–7].

We performed pancreatoduodenectomy for a PBM patient with simultaneous double cancer of the gallbladder and lower bile duct. To clarify the relationship between pathologic and genetic changes, we examined the p53 and the K-ras gene mutation and p53 protein overexpression in both carcinomas, the epithelium of the bile duct, pancreatic duct, common channel and the mucosa adjacent to the papilla of Vater.

Case report

A 77-year-old woman was admitted with jaundice on 20 March 2006. Abdominal computerized tomography (CT) and ultrasonography (US) showed intra- and extra-hepatic biliary duct dilatation and space occupying lesions in the gallbladder (Fig. 1a) and lower bile duct, respectively. Endoscopic retrograde cholangiopancreatography (ERCP) revealed an incomplete obstruction in the lower portion of the common bile duct. The cholangiopancreatography showed that the main pancreatic duct joined the bile duct 30 mm above the papilla of Vater and the main pancreatic duct joined the common bile duct (Type 1, or P–C union) [8] (Fig. 1b). We performed retrograde endoscopical biliary drainage and biopsies from a lower bile duct. Adenocarcinoma was detected in the biopsy specimens. Tumor markers, including carbohydrate antigen 19-9 (CA19-9), carcinoembryonic antigen (CEA) were within normal ranges. We diagnosed synchronous double cancers associated with PBM and performed pancreatoduodenectomy with lymph node dissection. Histological specimens revealed that the double cancers were not connected (Fig. 1c, d). The gallbladder lesion was papillary carcinoma (Fig. 1e) and that of the common bile duct was well differentiated tubular adenocarcinoma (Fig. 1f). We dissected 5 mm^2 fresh specimens from areas (A)–(F) of the resected specimens as shown in Fig. 2a. The specimens were divided into two parts; one was immediately frozen in liquid nitrogen and stored at −80°C for later DNA extraction and the other was chilled and fixed with Tissue-Tek freezing medium (Sakura Finetechnical Co., Ltd, Tokyo, Japan) for H&E and p53 immunohistochemical staining as described below. The remaining surgical specimens fixed in 10% formalin and embedded in paraffin for routine histological diagnosis. DNA was extracted from each frozen sample by the phenol–chloroform method and amplified using four pairs of forward and reverse primers labeled at 5′ end with Cy5 (Amersham Pharmacia Biotech, Piscataway, NJ, USA) for p53 gene analysis: exon 5: 5′-TTCCTCTTCCTACAGTACTCC and 5′-GCCCCAGC TGCTCACCATCGC, exon 6: 5′-CACTGATTGCTCT TAGGTCTG and 5′-AGTTGCAAACCAGACCTCAGG, exon 7: 5′-CCAAGGCGCACTGGCCTCATC and 5′-TCA GCGGCAAGCAGAGGCTGG, exon 8: 5′-CCTATCCTG AGTAGTGGTAAT and 5′-GTCCTGCTTGCTTACCTC GCT and TM High Fidelity PCR System (Roche Ltd, Basel, Switzerland). Electrophoresis of PCR products were done at 1,200 V for 5 h with ALF Express (Amersham Pharmacia Biotech, Piscataway, NJ, USA). A single-strand conformation polymorphism (SSCP) analysis was performed on analytic software Allele Link (Amersham Pharmacia Biotech). For extra peak of SSCP analysis, sequence was examined with a purified PCR product with a high pure PCR product purification kit (Roche), thermo sequenase fluorescent labelled primer cycle sequencing kit with 7-deaza-dGTP (Amersham Pharmacia Biotech,

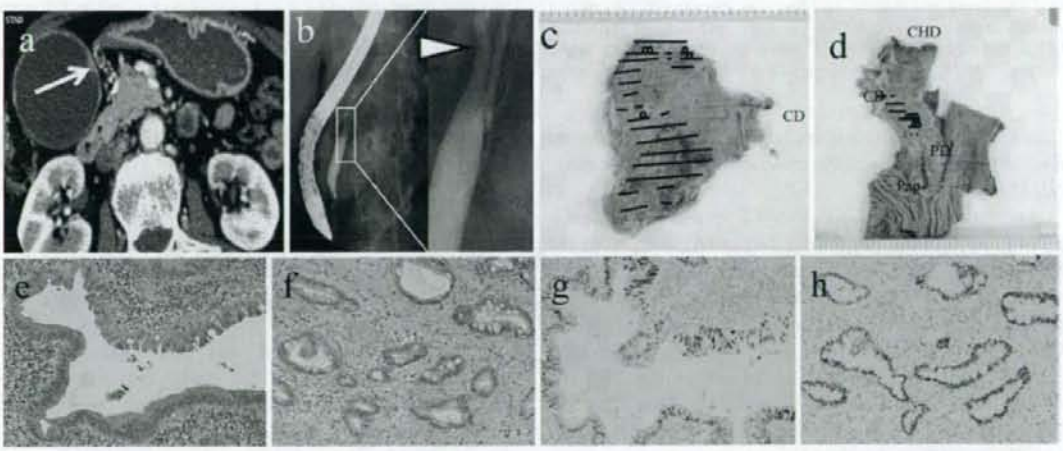

Fig. 1 Cancer of the gallbladder and the bile duct. **a** The elevated region (*arrow*) was shown in the lumen of gallbladder on CT. **b** Cholangiopancreatogram demonstrated a long common channel in PBM. The length of the common channel was 30 mm, and there was a narrow area (*arrowhead*) in the upper site of confluence of the bile duct and main pancreatic duct. **c** The histological mapping of the gallbladder cancer. *Solid line* mucosal carcinoma, *open circle* invasive cancer, *CD* cystic duct. **d** The histological mapping of the bile duct cancer. *Solid line* mucosal carcinoma, *open circle* invasive cancer. **e** H&E specimen of the gallbladder showed papillary carcinoma. *CHD* common hepatic duct, *CD* cystic duct, *PD* an open of the pancreatic duct, *Pap* papilla of Vater. **f** The specimen of the bile duct showed invasive well differentiated tubular adenocarcinoma. **g**, **h** p53 immunoreactivity was shown in the cancer of gallbladder and the bile duct

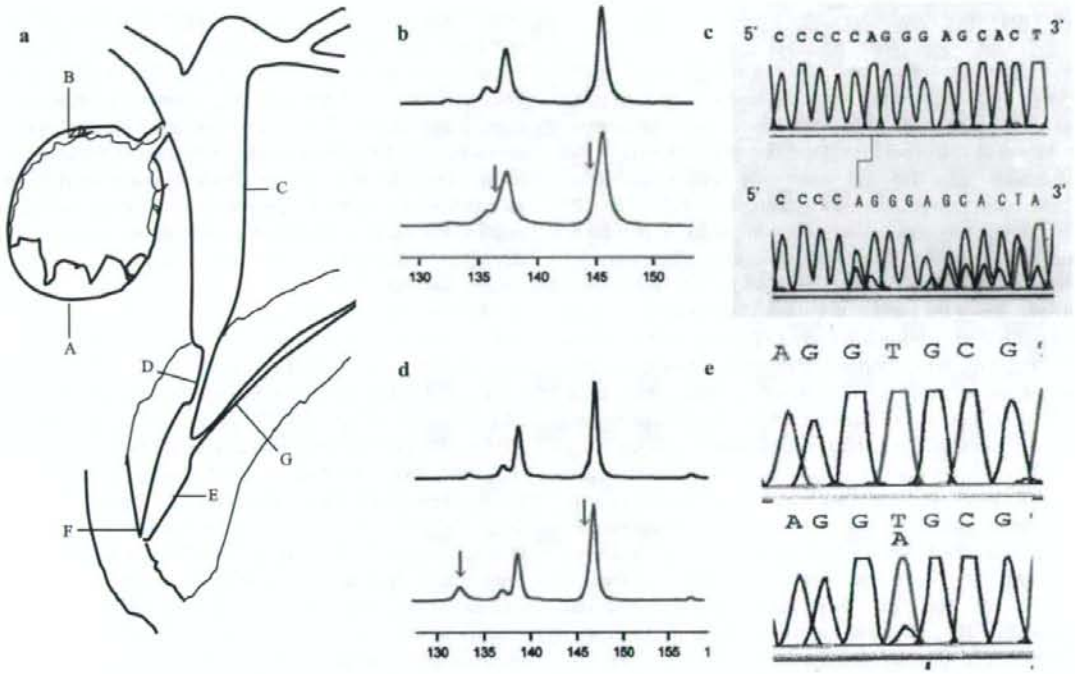

Fig. 2 **a** A schematic drawing of the resected specimen, which showed areas of sample collecting. A gallbladder carcinoma occupied the fundic portion of the gallbladder (*A*). Hyperplastic non-cancerous epithelium was presented at the neck and cystic duct of gallbladder (*B*). The upper and middle bile duct was extended by obstructive stenosis of lower bile duct lesion. There was no cancerous epithelium (*C*). Bile duct cancer occupied a limited area of the intra-pancreatic bile duct (*D*). Mild atypism was present in the common channel away from bile duct cancer (*E*). No atypical changes showed in the mucosa adjacent to the papilla of Vater (*F*) and main pancreatic duct (*G*). **b** Representative specimen of SSCP and direct sequencing of exon 8 of the p53 gene. Extra peaks on SSCP (*arrows*) and **c** non-sense mutation, frame shift of CCA–CA, was present on codon 301 in the gallbladder cancer. **d** In the bile duct cancer, extra peaks (*arrows*) and **e** missense mutation, GTG (*Val*) to GAG (*Glu*) was present on codon 272 in bile duct cancer (*C*)

Piscataway, NJ, USA) and analyzed by Analytic Software Evaluation (Amersham Pharmacia Biotech, Piscataway, NJ, USA).

An extra peak for p53 PCR-SSCP was shown in specimens from both the gallbladder cancer (A) and that of the bile duct (D). The non-sense mutation of CCA (*Pro*) to CA was found on codon 301 on exon 8 of the p53 gene in the gallbladder cancer (Fig. 2a) and the missense mutation of GTG (*Val*) to GAG (*Glu*) was found on codon 272 on exon 8 of the p53 gene in the gallbladder cancer (Fig. 2b). The other sites (B) (C) (E) (F) had no mutation of the p53 gene. No K-ras codon 12 gene mutation was shown at any sites (A)–(F) by PCR-preferential homoduplex formation assay.

To confirm whether the target cells were contained or not in these frozen specimens, the corresponding specimens for each DNA extraction were stained with H&E and p53 immunostaining. They were fixed in acetone and inactivated endogenous peroxidase with 3% hydrogen peroxide and non-specific antibody binding, and then, incubated with DO7 anti-human p53 mouse monoclonal antibody (Dako, Glostrup, Denmark), which recognizes both wild- and mutant-type p53 protein, for 10 min at room temperature. Excess antibody was washed out and incubated with secondary biotinylated anti-rabbit antibodies (LSAB2 Kit, Dako), avidin-biotin complex and 3,3′-diaminobenzidine. Specimens then were counterstained with hematoxylin.

p53 immunostaining was positive on specimens of the gallbladder cancer (A) (Fig. 1g), the lower bile duct cancer (B) (Fig. 1h) and the common channel area (E). Formalin fixed specimens were also used for p53 and Ki-67 (anti-human Ki-67 antibody MIB1, Immunotech, Marseille, France) immunohistochemistry. For p53 labeling, 70% or more of nuclei with an intensity that was clearly demarcated from surrounding mucosa at low power magnification were present in both the gallbladder and bile duct cancer. Moreover, p53 and Ki-67 immunoreactivity was shown on the epithelium of common channel (Fig. 3a–d).

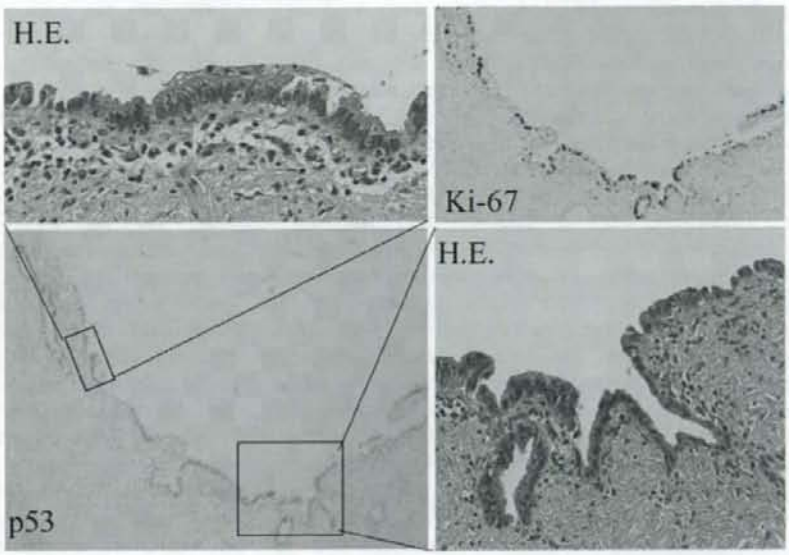

Fig. 3 Mild epithelial atypia was present in the common channel. Immunohistochemical staining showed many Ki-67-positive nuclei (*left-low*) and p53-positive nuclei

Discussion

The carcinogenetic process in PBM is caused by repeated damage and restoration of biliary epithelium by a mutual countercurrent of pancreatic and bile juice. Regenerated epithelium gradually produces a variant accompanied by cellular atypical change, displaying a hyperplasia–dysplasia–carcinoma sequence [9]. About 30% of patients with dilatation of the bile duct developed carcinogenesis not only in the gallbladder but also the bile duct [10]. Suzuki et al. [11] and Takayashiki et al. [12] reviewed 6 and 12 cases of multiple primary cancer of biliary tract associated with PBM, respectively. They proposed that carcinogenesis of the gallbladder and that of the bile duct were unrelated, on the basis of histological examination. Abnormalities of some oncogenes and cancer suppressor genes occur during each step of carcinogenesis [4–8]. Accumulation of gene abnormality was also important for carcinogenetic process in PBM, because a carcinogenic change was reported from a remnant bile duct or the pancreatic duct in a postoperative case [12]. Gene abnormality of PBM has received little attention. The present case with double primary cancer of the gallbladder and the bile duct is useful to clarify the genetic pathway of carcinogenesis in PBM. In addition, it is extremely valuable to pathologically examine and genetically evaluate abnormalities of the common channel.

The most prevalent K-ras mutation is shown in pancreatic cancer cells (80–100%) and/or metaplastic epithelium of pancreatic duct in cases of chronic pancreatitis [13]. It is suggested that K-ras mutation is an important factor of carcinogenesis related to pancreatic juice [14]. For examination of K-ras in gallbladder mucosa in PBM, K-ras mutation is strongly regarded as an early event in normal to hyperplastic epithelium [14, 15]. Hidaka et al. [14] reported the relation between K-ras mutation of gallbladder mucosa and length of the common channels in case without PBM. Mutation in non-cancerous epithelium was more frequent in long common channels (>5 mm) than in shorter ones. Furthermore, they gradually increased from the upper to lower bile duct. Their results suggested that both the location and the length of the common channel were linked to K-ras mutation and biliary carcinoma. Nagai et al. [16] also showed K-ras mutation in 36.4% of non-cancerous epitheliums of gallbladder, in 58.8% of gallbladder cancers, and in 20% of non-cancerous epithelium in biliary duct and in 62.5% of bile duct cancers. In the present case, the K-ras mutation was not observed at any site of cancer or non-cancerous epithelium. However, for epithelial cells in non-cancerous area with positive Ki-67, it might have caused another signal regulation apart from K-ras gene.

The p53 tumor-suppressor protein is considered to inhibit tumor growth and p53 mutation can be observed in the majority of malignant tumors [7, 8]. p53 induces p21, which causes cell arrest at the G1 period through phosphorylated RB protein, or causes cell death by apoptosis [17]. The most common mechanism of p53 inactivation is missense mutation within exons 5–8 [16]. It is thought that p53 mutation usually occurs in severe dysplasia (carcinoma in situ) or invasive cancer [17]. Matsubara et al. [18, 19] found p53 gene mutation in 75% of the gallbladder cancers and in 35.7 and 16.7% of non-cancerous lesions in the

gallbladder and common bile duct, respectively. Kamisawa et al. [20] also showed p53 protein overexpression in non-cancerous epithelium of the gallbladder in patients with a relatively long common channel. Both reports suggest that p53 gene mutations are involved early in the carcinogenesis of biliary epithelium. On the contrary, in the present case p53 gene mutation was recognized only in the gallbladder and bile duct cancer. The role of p53 mutation in the carcinogenesis of PBM requires further clarification.

Nagai et al. [16] reported p53 gene mutation of double cancer of the gallbladder and bile duct and mutation in gallbladder cancer but not in bile duct cancer. They, therefore, concluded that the carcinogenetic processes in the gallbladder and the bile duct differ. In the present study, both the gallbladder cancer and bile duct cancer showed the same pattern of p53 mutation without K-ras mutation. This did not agree with the result of Nagai et al. [16], but suggested that the genetic change may have involved the same pathway in the gallbladder and the bile duct, because the carcinogenic stimuli may be similar.

A relatively long common channel is thought to be an important risk factor for the development of gallbladder cancer [20]. However, reports on carcinogenesis in the common channel epithelium itself are rare, because almost all patients with PBM underwent cholecystectomy or choledochojejunostomy [2]. The structure of the papilla has many anatomic variations in PBM. We focused on the role of the common channel in PBM. Generally, a good correlation has been observed between the missense mutation and accumulation of p53 protein. Non-sense mutation sometimes shows a lack of p53 protein. However, the mutation was present on the exon 8 of p53 gene in the present case. The antibody (DO-7) recognized the epitope which was placed on the upper from the nonsense mutation of p53 gene. It reacts like an epitope in the N-terminus of P53 protein, known to reside between amino acids 35 and 45 [21]. In some cases, a discrepancy is observed when no mutations are detected in cancer cells with p53 protein overexpression [22]. The accumulation of wild-type protein in the present case might reflect a physiological response by p53 to such stimuli as DNA damage [23]. p53 protein accumulated in cells without p53 mutation in the common channel in the present case. If these cells were non-cancerous, they would usually undergo apoptosis; however, the up-regulation of the cell cycle was not related to wild type p53 status. We assumed that other cycle regulatory pathways might be more dominant than the p53 system in the Ki-67 positive epithelium. Cell proliferation is triggered by the binding of growth factors under physiologic conditions. As cell proliferation course except the course of p53, improving reputation of CyclinD [1, 24], TGF-α [25] and COX2 [26] were reported.

Clinically, carcinoma of the papilla of Vater is popular, but, as for the words called the common channel carcinoma, is not familiar. The papilla of Vater is composed of the common channel, the intraduodenal portion of the common bile duct, the intraduodenal portion of the pancreatic duct, and the duodenal mucosa [4]. The clinical cancer tends to occupied astride among them and cannot identify outbreak part closely. Therefore, the cancer of papilla of Vater is diagnosed collectively. Because a common channel in PBM is longer than that in PBM, it is compatible that an initiation of mutagenesis or carcinogenesis occurs in the common channel area widely. Furthermore, the high carcinogenic potential of common channel epithelium in PBM patients is required for part of the carcinogenetic process. According to Younes' report [27], the p53 accumulation in tumors of the papilla of Vater occurs early in the neoplastic process. Atypical epithelium was found most frequently in the common channel in patients with carcinoma of the papilla of Vater and this would seem to be related to the histological process involved in the development of biliary cancer in the dysplasia–carcinoma sequence [4, 9]. Additional molecular genetic studies are necessary to identify the carcinogenesis in PBM We should pay attention to not only the remnant intra-pancreatic bile duct but the common channel as a potential site for cancer in patients with PBM.

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Detection of circulating tumor cells in patients with pancreatic cancer: a preliminary result

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Abstract
Background/Purpose. It has been reported that circulating tumor cells (CTCs) can be used to predict survival in metastatic breast cancer. In this preliminary study, we examined the level of CTCs in pancreatic cancer (PC) patients to elucidate whether we could predict survival in PC.
Methods. The eligible subjects, at Tokyo Medical University Hospital, were 26 patients with PC, 11 with chronic pancreatitis, and 10 healthy volunteers. Three PC patients underwent surgery, 18 patients (who were stage IV) were treated with gemcitabine (GEM), and 5 patients received best supportive care (BSC).
Results. The CTC count was 1/7.5 ml blood or higher (defined as positive) in 11 of the 26 patients (42%; mean, 16.9/7.5 ml blood; range, 1–105/7.5 ml blood). Gemcitabine was administered to 6 of the 11 CTC-positive patients (3.8 courses on average). The treatment was continued for more than three courses in 2 patients, in both of whom the CTC count was only 1/7.5 ml blood. Operation was performed in 1 of the 11 CTC-positive patients. The remaining 4 patients of the 11 CTC-positive patients received only BSC. CTC was negative in 15 patients with PC (stage II, 1; stage III, 1; stage IVa, 7; and stage IVb, 6) and in the subjects with benign conditions. The median survival times (MSTs) of the CTC-positive and -negative patients were 110.5 and 375.8 days ($P < 0.001$). When the analysis was limited to the 14 stage-IVb patients, the MSTs of the CTC-positive and -negative patients were 52.5 and 308.3 days ($P < 0.01$).
Conclusions. The present study demonstrated that the detection of CTCs in peripheral blood may be useful to predict prognosis in patients with PC.

Key words CTC · Pancreatic cancer · Prediction of prognosis

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Introduction

Pancreatic cancer is the fifth leading cause of cancer death in Japan (The Editorial Board of Cancer Statistics in Japan. *Cancer statistics in Japan 2005*[1]), and it is usually unresectable (60%–70%) at the time of diagnosis, despite recent progress in imaging modalities.[2] Although the prognosis of not only advanced and metastatic pancreatic cancer but also even that of operable pancreatic cancer is poor, except in the fewer than 30% of patients in whom curative operation is possible, to date no prognostic markers have been established.[3] Circulating tumor cells (CTCs), which are one of these prognostic markers, can be detected in the peripheral blood of patients with cancer of all major organs, but not in healthy subjects or in patients with nonmalignant diseases.[4–11]

Recently, a new cell surveillance system, which can detect a small number of epithelial cells as a cancer marker, has been developed, and several investigators have described its efficacy as a prognostic marker in patients with carcinocythemia (cancer cell leukemia).[4,5,12–18] In the present preliminary study, we evaluated CTCs in blood obtained from patients with pancreatic cancer.

Patients and methods

Patients

Thirty-two patients with pancreatic cancer consulted our hospital between October 2004 and February 2006. Of these, 26 patients gave their written informed consent for the analysis of CTCs in peripheral blood. During the same period, 11 patients with chronic pancreatitis and 10 healthy volunteers were examined for CTCs. For the final diagnosis of the 26 pancreatic cancers, we obtained pathological evidence from surgical specimens in 3

patients and from endoscopic ultrasonography-guided fine-needle aspiration biopsy (EUS-FNAB) specimens in 16 patients. The remaining 7 patients refused biopsy, and diagnoses were made on the basis of diagnostic images. The 11 patients with chronic pancreatitis were diagnosed based on imaging studies and clinical follow-up for more than 12 months.

In the three surgically resected patients with pancreatic cancer, CTCs were examined preoperatively. All patients were treated with gemcitabine (Gemzal; Eli-Lilly, Indianapolis, IN, USA), 800 to 1000 mg/m^2 intravenously over 30 min, administered every 28 days on days 1, 8, and 22. In unresectable patients, chemotherapy using gemcitabine was performed unless the patients refused it or were in poor condition. Eligibility criteria for the study included Southwest Oncology Group performance status of 0–2; a life expectancy of greater than 12 weeks; and adequate bone marrow, hepatic, and renal function. This study was approved by the institutional review board of our institution.

Laboratory methods

Twenty milliliters of blood was drawn from patients with pancreatic cancer 2 weeks before any anticancer treatment such as surgery or chemotherapy. Blood samples were collected in ethylenediamine tetraacetic acid (EDTA)-containing Vacutainers (Becton Dickinson, Franklin Lakes, NJ, USA). Samples were maintained at room temperature and processed within 72 h after collection. The CellSearch system (Veridex LLC, Warren, NJ, USA) was used for the isolation and counting of CTCs. The CellSearch system consists of the CellTrack AutoPrep system, the CellSearch Epithelial Cell Kit, and the CellSpotter Analyzer. The CellTrack AutoPrep system is a semi-automated sample preparation system. The CellSearch Epithelial Cell Kit contains ferrofluids coated with specific epithelial cell adhesion molecule (EpCAM) antibodies to immunomagnetically enrich epithelial cells. Isolated cells were fluorescently labeled with the nucleic acid dye 4′, 6-diamidino-2-phenylindole (DAPI) and monoclonal antibodies specific for leukocytes (CD45-allophycocyanin) and epithelial cells (cytokeratin 8, 18, 19-phycoerythrin). Identification and enumeration were analyzed by the Cellspotter Analyzer. To be defined as a CTC, the object must be round or oval, have a nucleus (as determined by positive DAPI staining) contained within the cytoplasm (as determined by positive cytokeratin 8, 18, 19-phycoerythrin staining), and lack the expression of CD 45(as determined by negative CD45-allophycocyanin staining). Figure 1 shows CTC images from patients with pancreatic cancer. The CTCs are round to oval in shape, have an intact nucleus, show cytokeratin staining throughout the cytoplasm, and lack CD45 staining. Technical details of the CellSearch and CellSpotter Systems, including accuracy, precision, linearity, and reproducibility, have been described elsewhere.[12,19]

Efficacy assessment

Standard tumor response criteria were used to determine the objective tumor response. A complete response (CR) was defined as the disappearance of all measurable and evaluable disease for at least 4 weeks without the appearance of any new lesions. A partial response (PR) indicated a reduction of 50% or more in the sum of the products of the greatest perpendicular dimensions of all measurable lesions for at least 4 weeks without the appearance of any new lesions. Stable disease (SD) corresponded to a decrease of less than 50% in the sum of the products of the greatest perpendicular dimensions of measurable lesions or an increase of less than 25% in the sum of the products of the greatest perpendicular dimensions of measurable lesions for a minimum of 3 months. Progressive disease (PD) was defined as an increase of more than 25% in the sum of the products of the greatest perpendicular dimensions of measurable lesions, the appearance of new lesions, or deterioration of any evaluable disease. Survival was measured from the time of initiation of therapy until death. The response duration was defined as the time from response documentation to the first

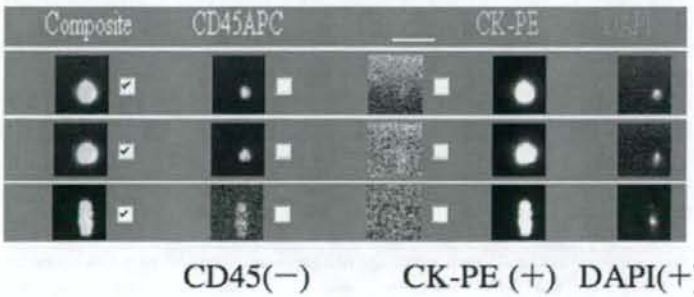

Fig. 1. Typical intact circulating tumor cell (CTC) images from the CellSpotter Analyzer (Veridex) obtained from patients with pancreatic cancer. *DAPI*, 4′, 6-diamidino-2-phenylindole; *CK-PE*, cytokeratin-phycoerythrin; *APC*, allophycocyanin

observation of progressive disease. At the time of analysis of CTCs, the tumor markers carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA) were examined.

Statistical analysis

The χ^2 test, when both variables were dichotomous, and Fisher's exact test were used to compare differences in categorical data between two groups, and the unpaired *t*-test was used to compare continuous data between two groups. Values are expressed as means (SD). A P value of less than 0.05 was considered significant. The Kaplan-Meier test was used for survival and time to progression. The log-rank test was applied to compare survival and time to progression between subgroups. A value of $P < 0.05$ was considered to indicate significance. All analyses were performed with the SPSS software package, version 10.0.5 (SPSS, Chicago, IL, USA).

Results

Patient characteristics

The clinical characteristics of the 26 patients with pancreatic cancer are shown in Table 1. The median age was 69.5 ± 13 years (range, 51 to 82 years); 15 patients were men. Twenty-two patients (85%) had a performance status of 0 or 1. The pancreatic masses were located in the head of the pancreas in 14 patients and in the body/tail in 12. Seven of the patients with masses in the head of the pancreas had obstructive jaundice. The stage of pancreatic cancer was: stage II in 1 patient, stage III in 1, stage IVa in 10, and stage IVb in 14. Metastatic cancer to the liver and lymph node were seen in both 11 patients, respectively. Regarding treatment, the 3 patients who underwent surgery had completely curative resections — pancreatoduodenectomy in 2 and pancreaticosplenectomy in 1. Eighteen of the 23 nonsurgical patients received chemotherapy with gemcitabine. These 18 patients received a median of 7.2 ± 4.6 cycles (range, 1–13 cycles) of chemotherapy. Of the remaining 5 patients, 2 did not agree to chemotherapy, and 3 had poor general conditions, such as ascites. CR was observed in none of the patients, PR was observed in 1 patient, and SD was observed in 14 patients, whereas 3 patients developed PD. Sixteen of the 26 patients with pancreatic cancer died and 10 were alive at the completion of the study. The overall mean survival time for all 26 patients was 7.8 months. The therapy was well tolerated, with grade 3–4 neutropenia (no grade 3–4 infection), thrombocytopenia (no bleed-

Table 1. Clinical characteristics of patients with pancreatic cancer

Patient no.	Sex	Age (years)	CTC	Stage TNM	T (mm)	N	M	PS	CA19-9 (U/ml)	CEA (ng/ml)	Therapy	Path	Outcome (days)
1	M	67	7	IVb	40	1	Liver	1	15000	6.4	Chemo	AD	98 Dead
2	F	78	5	IVb	35	—	Liver	1	50000	21.1	Chemo	NA	45 Dead
3	M	78	0	IVb	35	2	Liver	2	688	7.2	BSC	NA	31 Dead
4	F	79	105	IVb	35	2	Liver	3	50000	98.3	BSC	NA	5 Dead
5	F	76	7	IVb	30	1	Liver	1	189	6.1	BSC	NA	86 Dead
6	F	51	0	IVb	45	3	—	0	450	4.8	Chemo	AD	489 Dead
7	M	67	0	II	25	—	—	0	4.8	3.0	Op	AD	441 Alive
8	F	78	36	IVb	30	—	Liver	1	50000	7.9	Chemo	NA	75 Dead
9	F	82	0	IVa	30	—	—	1	9	9.0	Chemo	AD	430 Alive
10	M	72	0	IVa	30	—	—	0	141	3.0	Chemo	AD	321 Dead
11	M	55	0	IVb	30	1	Liver	0	925	4.4	Chemo	AS	446 Alive
12	M	74	0	IVa	35	—	—	1	119	3.0	Chemo	AD	452 Alive
13	M	70	0	III	30	1	—	0	127	7.4	Op	AD	462 Alive
14	F	65	0	IVa	35	—	—	0	359	3.6	Chemo	AD	462 Alive
15	F	67	0	IVa	35	—	—	1	1189	3.0	Chemo	AD	99 Dead
16	M	78	1	IVa	30	—	—	0	127	7.4	Chemo	AD	299 Dead
17	M	68	16	IVb	30	0	Peri	2	3122	6.8	Chemo	NA	57 Dead
18	F	58	0	IVb	30	3	—	0	9	9.8	Chemo	AD	305 Dead
19	M	78	2	IVb	35	—	Liver	2	890	8.8	BSC	NA	21 Dead
20	M	62	1	IVa	25	—	—	0	8.7	3.0	Chemo	AD	348 Alive
21	M	61	1	IVa	25	—	—	0	376	4.0	Op	AD	148 Dead
22	F	81	0	IVa	35	—	—	1	341	4.0	Chemo	AD	273 Alive
23	M	52	0	IVb	35	1	Liver	0	14246	5.2	Chemo	AD	108 Dead
24	M	69	0	IVa	35	—	—	1	2354	7.7	Chemo	AD	273 Alive
25	M	62	0	IVb	30	1	Liver	0	65	5.0	Chemo	AD	258 Alive
26	F	74	4	IVb	40	1	Liver	1	747	1.2	BSC	NA	33 Dead

PS, performance status; CTC, circulating tumor cell; Peri, peritoneal metastasis; Path, pathology; AD, adenocarcinoma; AS, adenosquamous carcinoma; NA, not available; Chemo, chemotherapy; Op, operation; BSC, best supportive care

ing), nausea, asthenia, and alopecia noted in 19.2%, 15.4%, 38.5%, 11.5%, and 8.0% of the patients, respectively. All 11 patients with chronic pancreatitis are currently alive.

CTCs in the blood of patients with pancreatic diseases

None of the control subjects or patients with chronic pancreatitis showed any CTCs per 7.5 ml of blood, giving a specificity of 100%. Based on these results, we considered even 1 CTC per 7.5 ml of blood to be abnormal, and we defined a CTC count of 1/7.5 ml of blood or higher as CTC-positive.

The median follow-up time of the patients with pancreatic cancer was 233.3 ± 170.9 days (range, 5–489 days). Eleven patients were CTC-positive (42%; 95% CI, 23%–63%; range, 1–105; mean, 16.9 ± 31.0/7.5 ml). One patient in stage II and one in stage III were CTC-negative. Three patients in stage IVa showed a CTC count of 1/7.5 ml blood. Eight of the 14 patients (57%) in stage IVb were CTC-positive (mean, 22.8 ± 35.0/7.5 ml blood).

The mean sizes of the pancreatic cancer tumors, measured by abdominal CT, were 33 mm in the 11 CTC-positive patients and 34 mm in the 15 CTC-negative patients, showing no significant difference. Invasion of the splenic artery/vein, portal vein, or superior mesenteric artery was noted in 10 of the 11 CTC-positive patients (91%). However, vascular invasion was also noted in 13 of the 15 CTC-negative patients (87%).

The mean CA19-9 level was 7394 ± 16185 U/ml (range, 5–50000 U/ml) in all patients; 15496 ± 22572 U/ml (range, 8.7–50000 U/ml) in the CTC-positive patients, and 1452 ± 3800 U/ml (range, 4.8–14246 U/ml) in the CTC-negative patients, showing a significant difference between the CTC-positive and CTC-negative patients ($P < 0.05$).

Correlation of positive CTC counts with clinical outcomes

Pancreaticosplenectomy was performed in 1 CTC-positive patient with cancer of the pancreatic tail (stage IVa), in whom the CTC count was 1/7.5 ml blood, but the patient died of peritoneal dissemination 148 days after surgery (patient 21 in Table 1). Six of the 11 CTC-positive patients received chemotherapy (3.8 courses on average). The treatment was continued for more than three courses in 2 patients (patients 16 and 20), in both of whom the CTC count was 1/7.5 ml blood. In contrast, in 4 patients who developed PD (patients 1, 2, 8, and 17), the CTC counts were 5, 7, 16, and 36/7.5 ml blood. The remaining 4 patients of the 11 CTC-positive patients did not receive chemotherapy due to their wishes, ascites retention, or complications, and the CTC counts were high: 2, 4, 7, and 105/7.5 ml blood, respectively (patients 4, 5, 19, and 26).

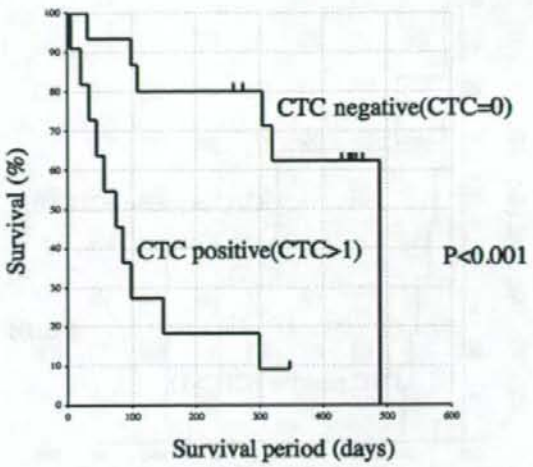

Fig. 2. Kaplan-Meier life-table analysis of the median survival time (calculated from the time of blood collection) in all patients with pancreatic cancer. The median survival times of the patients with pancreatic cancer were significantly different when stratified according to positive or negative CTC count

Correlation of negative CTC counts with clinical outcomes

Fifteen patients were CTC-negative (stage II, $n = 1$; stage III, $n = 1$; stage IVa, $n = 6$; and stage IVb, $n = 7$). Pancreatoduodenectomy was performed in 2 patients (1 stage II; 1 stage III) followed by adjuvant therapy with gemcitabine, and both patients are currently alive. Twelve of the 15 CTC-negative patients (stage IVa, 6; stage IVb, 5) received a median of 8.2 courses of chemotherapy (range, 2–13 courses), which was significantly different from the number of courses in the CTC-positive patients (3.8 vs 8.2 courses, $P < 0.05$). The therapeutic effects on CTC-negative patients were PR, NC, and PD in 1 patient, 13 patients, and 1 patient, respectively.

The median survival times (MSTs) of patients with positive and negative CTC counts were 110.5 days and 375.8 days, respectively (significantly different at $P < 0.001$; Fig. 2) In the 14 stage IVb patients, the MSTs of those with positive and negative CTC counts were 52.5 days and 308.3 days, respectively (significantly different at $P < 0.01$; Fig. 3).

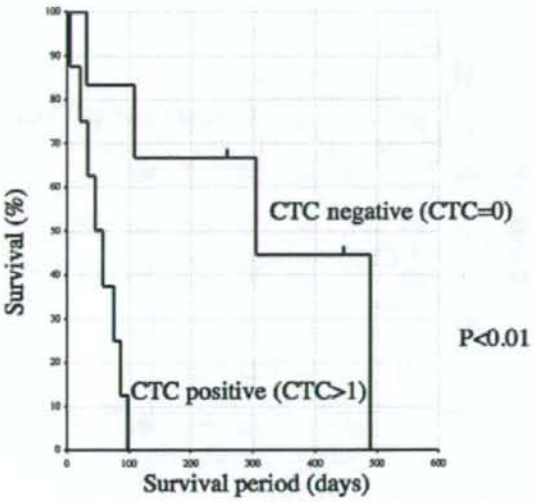

Fig. 3. Kaplan-Meier life-table analysis of the median survival time (calculated from the time of blood collection) in patients with stage IVb pancreatic cancer. The median survival times of the patients with stage IVb pancreatic cancer were significantly different when stratified according to positive or negative CTC count

Discussion

In this prospective study, we investigated the utility of analyzing CTCs in peripheral blood to predict the prognosis of patients with pancreatic cancer. The median survival time of CTC-negative patients was significantly longer than that of the CTC-positive patients. Therefore, this study showed that positivity for CTCs might affect survival. Interestingly, this hypothesis was also applicable to stage IVb patients with metastatic pancreatic cancers.

In the present study, CTCs were not seen in healthy subjects or in those with nonmalignant pancreatic diseases, only in the patients with pancreatic cancer. To date, although the methodology for measuring CTCs differs slightly in various laboratories, there have been several reports of CTCs in the peripheral blood of patients with many kinds of cancer.[4–19] The CellSearch system (Veridex), which can detect rare epithelial cells, has been developed recently, and, using this system, several investigators have reported that CTC detection might help to identify cancer patients with a poor prognosis.[4,5,14–19] Usually, the CTC count is extremely low (<2 CTCs per ml blood) or zero in healthy subjects and in patients with nonmalignant diseases.[4–19] In contrast, in many kinds of cancers, showed widely ranging CTC counts, from zero to more than 5000 per 7.5 ml blood.[4–19] In particular, research on CTCs is more advanced in breast cancer. Cristofanilli et al.[4] reported that a CTC count of more than 5 per ml blood could distinguish a favorable prognosis from an unfavorable one in patients with breast cancer. We suggest that a standard CTC count depends on the characteristics of each cancer. Allard et al.[19] described the result of investigating CTCs in peripheral blood in patients with 12 different types of metastatic cancers and reported that pancreatic cancers showed one of the lowest CTC levels (2 ± 6 CTCs per ml blood). We think that it is necessary to resolve this by understanding the anatomy of the pancreas. Reticuloendothelial systems, such as the liver and spleen, are present in bloodflow pathways invaded by pancreatic cancer, and trap cancer cells, reducing the possibility of cancer cells entering the systemic circulation. We think that stage IVb patients may have cancer cells in the circulation for at least some of the time, but not necessarily at all times. Because we performed detection of CTCs on only one occasion, it is possible that a sampling error occurred. However, we speculate that not all stage IVb patients continuously show sufficient amounts of cancer cells to be detected by the CellSearch System. In particular, in patients showing portal vein, superior mesenteric vein, or splenic vein invasion, many cancer cells may be trapped in the liver at an early stage, resulting in decreasing numbers of cancer cells in the systemic circulation. In fact, in the present study, all but three of the stage IVb patients showed liver metastasis and the hematogenous metastasis to the other organ was not shown. Therefore, we think that detection of CTCs may be useful even in patients showing stage IVb progression. A similar assumption regarding CTC count has been reported in colorectal cancer.[18] In the present study, CTC levels in patients with pancreatic cancer were low, but CTCs were absent in patients with nonmalignant pancreatic disease. To the best of our knowledge, there have been no reports on CTCs in patients with either benign or malignant pancreatic diseases, and thus further examination is necessary in the future.

Our results indicate that the presence of CTCs leads to a poor prognosis in pancreatic cancer, as well as in other cancers. Needless to say, many factors, such as individual conditions and effects of chemotherapy, are related to a poor prognosis, but surprisingly, a significant difference was noted in overall survival between our CTC-positive and CTC-negative patients in stage IVb, in whom prognosis is considered to be poor, despite there being no significant difference in the number of stage IVb patients receiving and not receiving chemotherapy. When we omitted from our analysis one stage IVb patient with performance status 3 (patient 5 and we examined the MSTs in the remaining 25 patients, the MSTs in those who were positive and negative for CTC counts were 121.0 days and 375.8

days, respectively ($P < 0.001$). In the remaining 13 stage IVb patients, the MSTs in those positive and negative for CTC counts were 59.3 days and 308.3 days, respectively ($P < 0.05$).

The reproducibility of the CellSearch system we used is high, and the accumulation of cases to validate our finding is necessary.

It is well known that when even locally advanced pancreatic cancer undergoes distant metastasis it may be dangerous. More than half of the patients with locally advanced cancer (stage IVa) in our study are alive at present; therefore, CTC as a prognostic factor in stage IVa patients could not be sufficiently investigated. Thus, CTC should be examined not only in patients with metastatic cancers but also in those with locally advanced cancers.

CA19-9 has been reported to be a factor determining the prognosis of pancreatic cancer, in terms of judging the therapeutic effect.[20] In the present study, a significant difference was noted in the CA19-9 levels between CTC-positive and CTC-negative patients. It is apparent that the prognosis is poor in patients with a CA19-9 level of 50000 U/ml or higher, but the level was not always elevated in patients with a poor outcome. Moreover, no clear cutoff values of CA19-9 are available to predict the prognosis. Thus, at present, it may be better to try to predict the prognosis based on CTC count alone or based on that count in combination with the CA19-9 level.

The surgical results of pancreatic cancer have been unsatisfactory, and some patients have postoperative local recurrence and distant metastasis. At present, no method is available to predict, before surgery, those who are likely to have postoperative distant metastasis. If postoperative distant metastasis could be predicted before surgery, it would surely improve the surgical results, and may help to maintain the patient's quality of life. Theoretically, the CTC count captures the phenomenon that occurs in the initial steps of distant metastasis. Improvements in the sensitivity of the measurement and in the blood sampling method, such as sampling volume, frequency, site, and intraoperative portal blood sampling, may enable the utilization of CTC counts for the prediction of postoperative distant metastasis in the near future.

In conclusion, although a larger series and longer follow-up are needed to confirm our data, the present study demonstrated that the detection of CTCs in peripheral blood may be useful to predict the prognosis in patients with pancreatic cancer.

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Personality and Colorectal Cancer: The Fukuoka Colorectal Cancer Study

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Objective: Although personality factors, especially emotional suppression and loss-hopelessness, have been linked to the occurrence and progression of cancer, little is reported specifically on colorectal cancer. It has also been claimed that a 'hysterical' personality characterized by exaggerated emotional expressions, egocentricity and ambivalent connection may be protective from cancer. This community-based case–control study examined whether personality factors relevant to emotional suppression or loss-hopelessness are associated with an increased risk of colorectal cancer, and whether factors related to the hysterical personality are associated with a decreased risk.

Methods: The stress inventory (SI), a self-administered questionnaire to assess the possible disease-prone and other relevant personalities in Japanese, was completed by 497 patients with newly diagnosed colorectal cancer and 809 controls randomly selected in the Fukuoka area of Japan.

Results: After controlling for age, sex and residence using a logistic regression model, none of the SI scales relevant to emotional suppression ('unfulfilled needs for acceptance', 'altruism', 'rationalizing conflicts/frustrations') or loss-hopelessness ('low sense of control', 'object-dependence/loss', 'object-dependence/happiness') was related to colorectal cancer. On the other hand, two scales representing elements of the hysterical personality, 'object-dependence/ambivalence' and 'egoism' were protectively associated with risk. Additional adjustment for body-mass index and lifestyle factors did not materially change these associations.

Conclusions: Although personalities relevant to the emotional suppression or loss-hopelessness may not be a risk factor for colorectal cancer in the Japanese population, ambivalent connection and egocentricity may be protective.

Key words: colorectal neoplasms – personality – stress, psychological – risk factors – case–control studies

INTRODUCTION

Chronic psychosocial stress is thought to affect lifestyle and the immune system (1,2), and may thus contribute to the development of cancer. Among personality factors thought to be prone to chronic stress, special attention has been focused on two: the reaction to a loss with chronic hopeless/helpless feelings (loss-hopelessness) and the suppression or inhibition of expressing negative emotions (emotional suppression) (3–7). Some epidemiologic studies have suggested that these factors may increase the risk of cancer of all sites (8–12), lung cancer (13) and breast cancer (14), although there are other studies failing to find such associations

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(15,16). Also, it has been claimed that the 'type 3' personality characterized by exaggerated emotional expressions, egocentricity and ambivalent connection may be protective from cancer (10,17). The type 3 personality is also referred to as 'hysterical' personality, a term which we used in this paper.

Colorectal cancer is one of the most common cancers in the world (18). Although the colorectum is known to be an organ sensitive to stress (19,20), few studies have specifically examined psychosocial factors in relation to colorectal cancer risk. Although some studies have suggested a possible role of job-related stress (21–23), life events (22,24), perceived stress (25) and social support (23) in the etiology of colorectal cancer, little is known for personality factors. To date, only two studies examined the association between personality factors and colorectal cancer, and they reported a positive association with aggressive hostility (26) and a personality profile relevant to emotional suppression (27).

The Fukuoka Colorectal Cancer Study was conducted to elucidate the role of lifestyle factors and genetic susceptibility in the etiology of colorectal cancer (28). In this community-based case–control study, participants completed the stress inventory (SI) (29–31), a self-administered questionnaire developed to assess the possible disease-prone personalities in Japanese. This paper addressed a hypothesis that the SI scales relevant to emotional suppression or to loss-hopelessness are associated with an increased risk of colorectal cancer and another hypothesis that the scales related to the hysterical personality are protectively associated with the risk.

PATIENTS AND METHODS

Methodological aspects in the design and conduct of the Fukuoka Colorectal Cancer Study have been described elsewhere (28). Briefly, cases were recruited from eight large hospitals in the study area (Fukuoka City and three adjacent area), and controls were randomly selected in the community by frequency matching to the distribution of incident cases with respect to sex and 10-year age class. The study protocol was approved by the Ethics Committees of Faculty of Medical Sciences, Kyushu University and of all but two of the participating hospitals, which did not have Ethics Committees at the time of survey.

Participants

The cases were a consecutive series of patients with histologically confirmed incident colorectal adenocarcinomas who were admitted to two university hospitals or six affiliated hospitals for surgical treatment. Other eligibility criteria were age of 20–74 years at the time of diagnosis, residence in the study area, no prior history of partial or total removal of the colorectum, familial adenomatous polyposis or inflammatory bowel disease, mental competence to give informed consent and to complete the interview. Research nurses contacted each eligible patient, and interviewed the patient who gave written informed consent. The nurses also asked the participating patients to complete the SI. During the survey period from October 2000 to December 2002, 544 of 669 eligible cases participated in the interview and 497 of the participants returned the SI. The participation rate was 74% (497 of 669).

Eligibility criteria for controls were the same as described for cases except for two items, i.e. having no diagnosis of colorectal cancer and age of 20–74 years at the time of selection. A total of 1500 persons were selected as control candidates by two-stage random sampling. The number of control candidates by sex and 10-year age class was determined in accordance to sex- and age-specific numbers of estimated incident cases of colorectal cancer. The first step was a random selection of 15 of 178 small areas, and then ~100 persons were randomly selected in each small area using the municipal resident registry on the basis of proportions of population in the small areas by sex and 10-year age class. A letter of invitation was sent to each candidate, and at most two additional letters of invitation were mailed to non-respondents. Of the 1500 candidates, 118 persons were excluded because of death ($n = 7$), migration from the study area ($n = 22$), undelivered mail ($n = 44$), mental incompetence ($n = 19$), history of partial or total removal of the colorectum ($n = 21$) and diagnosis of colorectal cancer after the survey ($n = 5$). Of the remaining 1382 persons, 833 participated in the interview survey, of which 809 returned the SI. The net participation rate was 59% (809 of 1382). The survey was carried out during the period from January 2001 to December 2002.

Research nurses interviewed the cases and controls in person regarding physical activity, smoking, alcohol use, parental history of colorectal cancer, dietary habit and others, using a validated, uniform questionnaire and instrument (32). The interview was done before or after the surgery at hospital wards for cases and at community halls and clinics for most of the controls; other places of interview for the controls were the work place, home and a university building.

Personality Assessment

The SI is a self-administered questionnaire used to assess the possible disease-prone and other relevant personalities in the Japanese population; its developmental procedures and psychometric properties (validity and reliability) are described elsewhere (29–31). Briefly, a pool of over 400 items was prepared with a special reference to the disease-prone/healthy personalities proposed by Grossarth-Maticek et al. (10,33). Starting with these items, a pilot series of interview surveys was done to obtain valid items using a variety of subjects including patients with cancer or myocardial infarction. Through this procedure, items were sorted and grouped into five, and a set of 75 items were revised and selected as appropriate for the SI (29). Based on factor analysis and correlation analyses with several conventional questionnaires, the SI was again shortened into 45 items (see Appendix 1),

and 12 scales were constructed (see Appendix 2); Cronbach alphas and test–retest reliability coefficients ranged from 0.60 to 0.90 and from 0.66 to 0.82, respectively (30).

In the present analysis, we focused on three scales related to emotional suppression and three scales relevant to loss-hopelessness. The three scales sharing emotional suppression as a common construct were 'unfulfilled needs for acceptance', 'altruism' and 'rationalizing conflicts/frustrations'. A high unfulfilled needs for acceptance score represents a situation where a person chronically has problems that he/she expects would be relieved if someone would listen, but his/her behaviors of telling such problems to others are usually suppressed and blocked. A person with a high altruism score tends to fill others' needs first, suppressing his/her own needs. A person with a high rationalizing conflicts/frustrations score would never vent his/her anger to others, rationalizing such feelings instead. 'Low sense of control', 'object-dependence/loss' and 'object-dependence/happiness' are the scales relevant to loss-hopelessness. A person with a high 'low sense of control' score, i.e. who loses the sense of control over stressful situations, would become hopeless easily. 'Object-dependence' refers to a tendency to have an object (person or condition) on which one's well-being is greatly dependent (10), and object-dependence/loss indicates that one tends to have hopeless and depressive feelings in the relationship with such an object. Object-dependence/happiness was developed as an antecedent condition in which one is apt to experience chronic hopeless feelings when encountering an important loss, and it may also be indirectly related to loss-hopelessness. We also focused on two scales, 'object-dependence/ambivalence' and 'egoism', which represented elements of the hysterical personality. The hysterical personality is characterized by exaggerated emotional expressions, egocentricity and ambivalent connection and has been suggested to be protective from cancer (10,17). A high object-dependence/ambivalence score indicates that one repeatedly experiences 'ambivalent' interpersonal relationships; an ambivalent person here refers to one who oscillates between idealizing an object (typically a person) which is being sought and attributing negative values to the object, devaluing it and seeking to escape from it. The egoism scale measures a self-defensive, self-interest-oriented attitude in interpersonal and social relationships. The SI answers were a six-point rating, 1–6, where 1 and 6, respectively, correspond to 'yes' and 'no', or to 'almost always' and 'rarely'. The score of each scale is the average of corresponding item scores and ranges between 1 and 6.

Statistical Analysis

The association of personality factors with the risk of colorectal cancer was examined in terms of odds ratio (OR) and 95% confidence interval (CI). The SI scale scores were categorized using quartiles in the distribution of the controls. Adjusted ORs were estimated from multiple logistic regression analysis, including indicator variables for gender, 5-year age class (the lowest class of <35 years) and area of residence (Fukuoka City or suburban area) as covariates. Trend of association was assessed with ordinal scores 1–4 assigned to four categories in order. In addition, the following anthropometric and lifestyle factors were also considered as potential confounders: body-mass index (BMI) at 10 years before (<25 or ≥25 kg/m^2), smoking (0, 1–399, 400–799 or ≥800 cigarette-years), alcohol intake (0, 0.1–0.9, 1.0–1.9, or ≥2.0 U/day), type of job (sedentary or non-sedentary), non-job physical activity (0, 1–15.9 or ≥16 MET-h/week), vegetables intake (tertiles), fruit intake (tertiles), red meat intake (tertiles) and fish intake (tertiles). All *P*-values were two-sided and considered significant at $P < 0.05$. All analyses were done using the SAS (version 9.1; SAS Institute Inc., Cary, NC, USA).

RESULTS

Of the patients and controls, 288 (58%) of 497 and 502 (62%) of 809, respectively, were men. The respective mean ages (range) of the cases and controls were 60 (29–74) and 59 (22–75) years, respectively. Residents in Fukuoka City accounted for 61% of the cases and 65% of the controls. Two hundred and seventy nine (56%) and 207 (42%) cases had cancer at colon and rectum and the remaining 11 cases (2%) had cancer at multiple sites. Because the cases were patients undergoing surgery, advanced disease was relatively uncommon; stages III (tumor invasion to nearby organs or metastasis to lymphnodes) and IV (metastasis to distant organs) according to the 1992 TNM Classification of Malignant Tumors (International Union Against Cancer) (34) accounted for 34 and 13%, respectively. Analysis of covariance including sex, age and residence as covariates found that some of the SI scales were associated with either or both sex and age, and the pattern of association was similar between cases and controls. Notably, women tended to score higher on disclosure of negative emotions and rationalizing conflicts/frustrations and lower on egoism than men. Older persons tended to score lower on object-dependence/happiness, annoying barrier and disclosure of negative emotions, and higher on lacking emotional experiences than younger persons (data not shown).

Table 1 shows the association between colorectal cancer risk and the scales related to emotional suppression. Controlling for sex, age and resident area, none of the three relevant scales (unfulfilled needs for acceptance, altruism and rationalizing conflicts/frustrations) was positively associated with colorectal cancer risk. Additional adjustment for BMI at 10 years before and lifestyle factors including smoking, alcohol consumption, physical exercise and dietary habits did not change such associations. Table 2 shows the association with the personality scales related to loss-hopelessness. Object-dependence/happiness tended to be positively associated with colorectal cancer, but the association

was statistically barely significant with a trend $P = 0.053$. The other two, low sense of control and object-dependence/loss, were unrelated to risk. These results did not change after controlling for BMI and lifestyle factors. On the other hand, both of the scales related to the hysterical personality (object-dependence/ambivalence and egoism) were each significantly, inversely associated with colorectal cancer risk (Table 3). Adjustment for BMI and lifestyle factors changed these associations little. None of the other scales showed a clear pattern of an association with colorectal cancer, either with or without adjustment for BMI and lifestyle factors. The multivariate-adjusted OR (95% CI) of the highest versus lowest scale score was 1.12 (0.82–1.53) for object-dependence/anger, 1.19 (0.84–1.69) for annoying barrier, 0.95 (0.67–1.34) for disclosure of negative experiences and 0.87 (0.63–1.22) for lacking emotional experiences.

When we analyzed the data separately for earlier disease (stages 0, I, II) and advanced disease (stages III, IV), the inverse associations of objective dependence/ambivalence and egoism were suggested to be somewhat stronger in the latter than in the former. The multivariate-adjusted ORs (95% CI) of the highest versus lowest score of object-dependence/ambivalence were 0.69 (0.45–1.06) for earlier

Table 1. The association between colorectal cancer and personality scales relevant to emotional suppression

Personality	*N*		Age, sex, and residence-adjusted OR (95% CI)	Multivariate-adjusted[1] OR (95% CI)
	Cases	Controls		
Unfulfilled needs for acceptance				
Q1	121	195	1.00 (referent)	1.00 (referent)
Q2	130	205	1.04 (0.76–1.44)	1.04 (0.75–1.44)
Q3	125	200	1.04 (0.75–1.43)	1.07 (0.77–1.49)
Q4	104	195	0.87 (0.62–1.22)	0.86 (0.61–1.21)
Trend			$P = 0.44$	$P = 0.46$
Altruism				
Q1	116	187	1.00 (referent)	1.00 (referent)
Q2	110	199	0.90 (0.65–1.25)	0.90 (0.64–1.26)
Q3	138	201	1.14 (0.83–1.58)	1.17 (0.85–1.63)
Q4	120	210	0.90 (0.65–1.24)	0.88 (0.63–1.23)
Trend			$P = 0.88$	$P = 0.85$
Rationalizing conflicts/frustrations				
Q1	133	198	1.00 (referent)	1.00 (referent)
Q2	123	222	0.84 (0.61–1.15)	0.83 (0.60–1.14)
Q3	116	213	0.82 (0.59–1.13)	0.81 (0.58–1.12)
Q4	109	161	0.99 (0.72–1.39)	1.00 (0.71–1.41)
Trend			$P = 0.84$	$P = 0.87$

Q1–Q4, quartile 1 (low)–quartile 4 (high); OR, odds ratio; CI, confidence interval; BMI, body-mass index at 10 years before. [1]Adjusted for BMI, smoking, alcohol consumption, type of job, non-job physical activity, vegetables, fruit, red meat and fish, as well as for age, sex and residence.

Table 2. The association between colorectal cancer and personality scales relevant to loss-hopelessness

Personality	*N*		Age, sex and residence-adjusted OR (95% CI)	Multivariate-adjusted[1] OR (95% CI)
	Cases	Controls		
Low sense of control				
Q1	128	207	1.00 (referent)	1.00 (referent)
Q2	93	170	0.87 (0.62–1.22)	0.90 (0.64–1.26)
Q3	135	217	1.00 (0.73–1.36)	1.02 (0.74–1.40)
Q4	129	201	1.01 (0.74–1.39)	1.01 (0.73–1.39)
Trend			$P = 0.75$	$P = 0.80$
Object-dependence/loss				
Q1	100	181	1.00 (referent)	1.00 (referent)
Q2	147	205	1.30 (0.93–1.80)	1.28 (0.92–1.79)
Q3	109	206	0.96 (0.69–1.36)	0.95 (0.67–1.34)
Q4	124	205	1.12 (0.80–1.57)	1.12 (0.79–1.57)
Trend			$P = 1.00$	$P = 0.96$
Object-dependence/happiness				
Q1	124	229	1.00 (referent)	1.00 (referent)
Q2	106	188	1.10 (0.79–1.52)	1.08 (0.77–1.51)
Q3	123	199	1.16 (0.85–1.60)	1.20 (0.87–1.65)
Q4	126	178	1.38 (1.00[2]–1.91)	1.37 (0.99–1.91)
Trend			$P = 0.05$[3]	$P = 0.05$[3]

[1]Adjusted for BMI, smoking, alcohol consumption, type of job, non-job physical activity, vegetables, fruit, red meat and fish, as well as for age, sex and residence. [2]Less than unity. [3]Greater than 0.05.

Table 3. The association between colorectal cancer and personality scales relevant to hysterical personality

Personality	*N*		Age, sex and residence-adjusted OR (95% CI)	Multivariate-adjusted[1] OR (95% CI)
	Cases	Controls		
Object-dependence/ambivalence				
Q1	140	197	1.00 (referent)	1.00 (referent)
Q2	126	206	0.86 (0.63–1.17)	0.87 (0.63–1.19)
Q3	133	210	0.90 (0.66–1.23)	0.90 (0.66–1.24)
Q4	85	183	0.67 (0.48–0.94)	0.66 (0.47–0.93)
Trend			$P = 0.04$	$P = 0.04$
Egoism				
Q1	142	214	1.00 (referent)	1.00 (referent)
Q2	174	249	1.08 (0.80–1.44)	1.06 (0.79–1.43)
Q3	103	177	0.96 (0.69–1.35)	0.98 (0.70–1.37)
Q4	59	153	0.62 (0.43–0.90)	0.59 (0.40–0.86)
Trend			$P = 0.02$	$P = 0.01$

[1]Adjusted for BMI, smoking, alcohol consumption, type of job, non-job physical activity, vegetables, fruit, red meat and fish, as well as for age, sex and residence.

disease and 0.62 (0.40–0.99) for advanced disease, and those of egoism were 0.63 (0.39–1.02) for earlier disease and 0.50 (0.44–1.12) for advanced disease.

DISCUSSION

Emotional suppression is a personality factor that has long been linked to cancer-proneness (3–7). Although not all (15,16), some epidemiologic studies have supported this notion in breast cancer (14), lung cancer (13) or cancer of all sites (8–10,12). This construct is referred to as several aspects using different terms, such as 'repression' (8,15,16), 'rationality/anti-emotionality' (13,14) or 'type 5' personality (10), 'loner and emotional suppression' (9), 'type C' personality (35), 'type 1' personality (10) and 'emotional control' (12,14). In an Australian community-based case–control study, Kune et al. (27) found an increased risk of colorectal cancer associated with a personality profile, characterized by repression, denial, non-expression of anger, social desirability, conflict avoidance and the suppression of reactions that may offend others. The three SI scales (unfulfilled needs of acceptance, altruism and rationalizing conflicts/frustrations) shared emotional suppression as an essential element, but the present study did not support the hypothesis that emotional suppression increases colorectal cancer risk.

Loss-hopelessness is another 'traditional' cancer-prone personality (3,6,7). A population-based cohort study in Finland reported that a high score on the 'hopelessness scale' was predictive of subsequent cancer of all sites (11). To our knowledge, no study has addressed this issue specifically for colorectal cancer. In the present study, the two scales relevant to loss-hopelessness, low sense of control and object-dependence/loss were not associated with an increased risk of colorectal cancer. The scale 'object-dependence/happiness' refers to a tendency to have a highly valued person on whom one's happiness is greatly dependent. Thus, this scale was thought to be an antecedent condition from which one is apt to experience chronic hopeless feelings when encountering an important loss, leading to the characteristics represented by the object-dependence/loss scale. In this sense, it is not straightforward to interpret the results indicating that object-dependence/happiness, not object-dependence/loss, was associated with colorectal cancer. The observed association with object-dependence/happiness might suggest that after notification of cancer diagnosis, the patients had become more aware of the importance of the support from their partner or other persons and realized that such support was necessary to their happiness.

Egoism and object-dependence/ambivalence represent elements of the hysterical personality, which Grossarth-Maticek et al. (10) proposes to be resistant against cancer. This study supported the hypothesis that these scales are protectively associated with colorectal cancer. An egocentric tendency may be of merit as a self-defense mechanism in interpersonal relations, leading in turn to the maintenance of health. The egoism scale was originally developed as the opposite of the altruism scale (29,30), but the latter was not related to colorectal cancer here. Egoism and altruism were, however, only weakly, if any, negatively correlated with each other in the present sample (Pearson correlation coefficients were −0.02 and −0.18 in the cases and controls, respectively). Because a highly ambivalent tendency should lead to the instability of feelings, it would be rather odd if such a characteristic were to favor cancer prevention. No studies have reported explicitly on ambivalence in relation to cancer, but some have found that 'worry' (36), 'anxiety' (14) or 'having anxiety disorder' (37), which may represent the instability of feelings, was unrelated to cancer risk. Ambivalence as a construct of the hysterical personality refers to a special form of instability in interpersonal relationships. Thus, an 'ambivalent' person oscillates between the two opposite aspects of object-dependence, sometimes idealizing an object (typically a person) which is being sought and sometimes attributing negative values to the object that he/she seeks to escape from. The former aspect corresponds to the concept represented by the object-dependence/loss and object-dependence/happiness scales, which are cancer-prone, and the latter corresponds to the concept represented by the object-dependence/anger and annoying barrier scales, which are coronary heart disease-prone (see Appendix 2). Grossarth-Maticek et al. (10) argues that ambivalence may protect one to some extent from the build-up of behavior patterns related to cancer or coronary heart disease.

The present study is subject to several drawbacks derived from its retrospective design. First, the majority of the cases had been informed of their cancer diagnosis before the time of the interview or questionnaire administration. It is possible that self-reported personality traits change after cancer diagnosis (38), and thus the differences in personality features between the cases and controls observed in the present study might be due to the psychological impact on the patients of the cancer diagnosis. In addition to the above discussed interpretation for the association with object-dependence/happiness, we cannot preclude the possibility that the patients tended to become less ambivalent and less egoistic after diagnosis. Second, the net participation rate was higher for the cases (74%) than for the controls (59%). It is possible that the cases included more submissive or altruistic persons and fewer selfish or egoistic persons. Such bias has the potential to mask the true association between altruism and colorectal cancer and to attenuate the observed association with egoism. Another limitation of the study was relevant to the instrument used for assessing personalities. Besides the elements represented by object-dependence/ambivalence and egoism, the construct of the hysterical personality involves another major element, 'inappropriate and exaggerated expression of feelings' (10). Because the SI does not include a scale representing this element, we could not address the question if this element was also inversely associated with colorectal cancer.

Two processes are thought to mediate the link between psychosocial factors and the development of cancer (1). Chronic

stress or personality susceptible to chronic stress are associated with unfavorable lifestyle factors, such as smoking, alcohol consumption, lack of physical activity, obesity and unhealthy eating (39–42). In the control subjects of the present study, some of the personality scores were significantly correlated with anthropometric and lifestyle factors (data not shown). For example, the low sense of control score was negatively correlated with red meat consumption, egoism was negatively correlated with fruit intake and rationalizing conflicts/frustrations was positively correlated with fruit intake. However, correlations between the individual personality scales and lifestyle factors were generally weak (the abstract values of Spearman's rank correlation coefficient <0.1), and the associations between these personalities and colorectal cancer were not altered by the adjustment for lifestyle factors and BMI. Other mechanisms to be considered include those involving psychophysiological processes. Chronic stress may have an impact on the neuro-endocrino-immunological network that may lead to the development of cancer via immunosuppression (2). The present data suggested that the inverse associations of colorectal cancer with ambivalence and egoism, especially the latter, were stronger for advanced disease than earlier disease. This may indicate that chronic stress is more closely related to the progression of clinical cancer than of subclinical cancer or the precancerous stage. However, we have not studied whether ambivalence, egoism or other relevant personality factors are associated with anti-cancer immune activities.

The present study is a community-based study of a fairly large scale and is among the few studies that have specifically addressed the role of personality factors on the development of colorectal cancer (26,27). We addressed an *a priori* hypothesis regarding personality as a risk factor for colorectal cancer, used a healthy community sample as controls, used validated instruments for assessing lifestyle and personality factors, and controlled for known or potential confounding factors. In addition, this is the first study suggesting the possible protective role of ambivalence and egoism on the etiology of colorectal cancer.

In conclusion, the present study did not support the hypothesis that personalities related to emotional suppression or loss-hopelessness are associated with colorectal cancer, although a possible positive association with object-dependence/happiness was not rejected. On the other hand, it suggests that ambivalent connection and egocentricity can be protective against colorectal cancer. These findings warrant further investigation.

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Conflict of interest statement

None declared.

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