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Clinical impact of circulating tumor cells and therapy response in pancreatic cancer

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Abstract

Background: Among gastrointestinal cancers, the prognosis of pancreatic cancer is one of the poorest, with a large number of patients being diagnosed with unresectable tumors at the first visit to a doctor. The aims of the present study were to investigate the circulating tumor cells (CTC) in peripheral blood in order to assess their clinical significance in patients with pancreatic cancer.
Methods: Sixty-five patients with advanced pancreatic cancer were enrolled. Borderline resectable pancreatic tumor patients were 9, and Unresectable patients were 56. The CellSearch system was used to isolate and enumerate CTCs.
Results: CTCs were identified in 21 out of 65 patients (32.3%) with only unresectable tumors. The overall survival rate was significantly lower in unresectable patients with than in those without CTCs ($P = 0.0051$). CTC positivity was significantly higher in patients with than in those without liver metastasis. A multivariate analysis identified the presence or absence of CTCs as an independent prognostic factor. Follow-up blood specimens were obtained from 40 patients treated with chemotherapy or chemoradiotherapy. The incidences of CTC positivity at three months after beginning of treatments in patients with progressive disease and stable disease or a partial response were 45.4% and 24.1%, respectively. The overall survival rate was significantly lower in patients with than in those without CTCs even after treatments ($P = 0.045$).
Conclusion: CTC numbers represents a useful tool for predicting prognoses and therapeutic responses to chemotherapy among patients with advanced pancreatic cancer.

Keywords: Circulating tumor cells; Liquid biopsy; Pancreatic cancer; The CellSearch system; Chemotherapy and chemoradiotherapy

Introduction

Pancreatic cancer is one of the most aggressive malignancies in gastrointestinal cancers, with most patients already having unresectable tumors at the first visit to a doctor. Moreover, patients with resectable pancreatic cancer develop recurrence in the early postoperative period. Recent developments in chemotherapy and chemoradiotherapy (CRT) have contributed greatly to improving the prognosis of advanced pancreatic cancer.[1] However, it is clinically difficult to predict therapeutic effects and prognoses using pre-therapeutic imaging examinations and conventional serum tumor markers, such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9).

The detection of circulating tumor cells (CTCs) in peripheral blood specimens has been attempted using various molecular biological approaches, such as a reverse transcriptase-polymerase chain reaction (RT-PCR) and flow cytometry.[2–5] Previous studies demonstrated that the evaluation of CTCs in pre-treatment blood was useful for predicting tumor progression and prognoses in patients with various malignancies including pancreatic cancer.[6–8] Most studies have assessed CTCs in peripheral blood

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specimens collected from patients with pancreatic cancer using the RT-PCR approach. We also reported the clinical significance of CTCs based on an RT-PCR assay for the expression of CEA mRNA.[9–11]

We previously demonstrated the usefulness of the CellSearch system in predicting tumor prognosis and therapeutic effects to chemotherapy or CRT by monitoring CTCs in patients with esophageal squamous cell carcinoma and gastric cancer.[12,13] The CellSearch System (Janssen Diagnostics, LLC, Raritan, NJ, USA) has been developed to identify CTCs in blood specimens and its clinical utility has already been reported in patients with several cancers, such as metastatic breast, prostate, and lung cancers.[6,14–17] However, the clinical significance of CTC monitoring using the CellSearch system has not yet been determined in the blood specimens of patients with advanced pancreatic cancer.

The aims of the present study were to investigate CTCs in the blood specimens of patients with advanced pancreatic cancer using the CellSearch system and assess the relationship between the presence or absence of CTCs and tumor properties, including prognosis and tumor responses to chemotherapy or CRT, in these patients.

Patients and methods

Patients

Patients with advanced pancreatic cancer who were treated at Kagoshima University Hospital were analyzed using prospectively collected data. Informed consent was obtained from all patients in accordance with the ethical standards of the Committee on Human Experimentation of Kagoshima University Hospital.

Sixty-five consecutive untreated patients were enrolled between August 2011 and May 2015. All patients were assessed by blood tests including serum tumor markers (CEA and CA 19-9), endoscopic ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), and ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) before the initiation of treatments. Patients were grouped and staged based on the tumor-node-metastasis (TNM) classification for pancreatic cancer established by the International Union Against Cancer (UICC). Table 1 shows clinicopathological factors. The number of patients in each clinical stage was 9 cases in II, 16 cases in III, 40 cases in IV, respectively. Borderline resectable pancreatic tumor patients were 9, and Unresectable patients were 56. Hematogenous metastases and peritoneal dissemination were identified in 28 and 16 patients. CTCs were evaluated before the initiation of chemotherapy or CRT in all patients.

Table 1
Characteristics of patients with pancreatic cancer (n = 65).

	Total (65)	
Gender	Male/Female	33/32
cT factor	2/3/4	1/13/51
cN factor	0/1	31/34
cM factor	0/1	25/40
Clinical stage	II/III/IV	9/16/40
	BR/UR	9/56
Serum CEA	Negative/Positive	42/23
Serum CA19-9	Negative/Positive	13/52

Treatment and evaluation of tumor responses

We assessed quantitative variations in CTCs before and at three months after beginning of treatments in 40 patients treated with chemotherapy or CRT. In the present study, 30 patients received chemotherapy and 10 patients CRT. CRT consisted of chemotherapy by S-1 (Taiho Pharmaceutical Co., Ltd., Tokyo, Japan) administered orally twice daily at a dose of 80 mg/m^2/day for 3 weeks and concomitant radiation therapy at a total dose of 50–58 Gy. In chemotherapy, S-1 and/or gemcitabine (GEM) (Nippon Kayaku Co., Ltd., Tokyo, Japan) was administered at a dose of 800–1500 mg/m^2/day twice for 21 days as a first-line regimen.

Tumors were evaluated using computed tomography (CT) examination every 3 month and tumor responses were determined by the Response Evaluation Criteria in Solid Tumors (RECIST guideline version 1.1).[18] Post-therapeutic blood specimens were collected to assess CTCs using the CellSearch system. The median follow-up period in this group was 19.8 months (range 2–58.3 months). Overall Survival was determined from the date of starting treatment to the date of death or last follow-up.

Detection of CTCs using the CellSearch system

Ten milliliters of blood was drawn into a CellSave Preservative Tube (Janssen Diagnostics, LLC). Blood specimens were maintained at room temperature and processed within 72 h of being collected. All blood processing was performed by technical assistants without knowledge of the clinical backgrounds of the patients. The CellSearch system was used to isolate and enumerate CTCs. A total of 7.5 ml of the 10 ml collected in tubes was evaluated by the system. The CellSearch system consists of a semi-automated system for the preparation of a sample and is used with the CellSearch Epithelial Cell Kit. The procedure enriches the sample for cells expressing epithelial cell adhesion molecule (EpCAM) with antibody-coated magnetic beads, and it labels the nucleus with the fluorescent nucleic acid dye 4, 6-diamidino-2-phenylidole dihydrochloride (DAPI). Fluorescently-labeled monoclonal antibodies specific for leukocytes (CD45-allophycocyan) and epithelial cells (cytokeratins 8 and 19 and 19-phycoerythin) are used to distinguish epithelial cells from leukocytes. The identification and enumeration of CTCs were performed using Celltracks analyzer II, a semi-automated fluorescence-based microscopy system that

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