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ORIGINAL ARTICLE

Clinical potential of the anticancer drug sensitivity test for patients with synchronous stage IV colorectal cancer

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Abstract
Purpose We retrospectively evaluated the clinical efficacy and feasibility of a collagen gel droplet-embedded culture drug sensitivity test (CD-DST) to guide therapy for patients with stage IV colorectal cancer (CRC).
Methods We investigated 38 patients with stage IV CRC. All patients were younger than 85 years and had untreated evaluable metastatic lesions. The primary tumors were surgically resected, and the tissue samples were investigated by CD-DST. Patients treated with in vitro sensitive drugs were defined as Group A ($n = 14$), while those treated with in vitro non-sensitive drugs were defined as Group B ($n = 24$). We evaluated response rate (RR), progression-free survival (PFS), and overall survival (OS).
Results RR was 85.71 % in Group A and 41.67 % in Group B ($p = 0.0079$). The median PFS was 696.5 days in Group A and 297.5 days in Group B ($p = 0.0326$). The median OS was 1,023.4 days in Group A and 518.5 days in Group B ($p = 0.0061$).
Conclusions The CD-DST can define chemoresistant and chemosensitive tumors. The use of CD-DST might be one of the tools to supplement informed consent prior to initiation of therapy.

Keywords Stage IV colorectal cancer · Anticancer drug · Sensitivity · CD-DST

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Introduction

Colorectal cancer (CRC) is one of the leading causes of death worldwide and continues to increase in incidence. Of patients with newly diagnosed CRC, 15–25 % have metastatic disease, which is usually lethal [1]. Furthermore, 50 % or more of the patients who are initially diagnosed with localized disease ultimately develop stage IV CRC [2]. The main treatment for stage IV CRC is chemotherapy, and recent advances in systemic chemotherapy have resulted in improved outcomes for these patients. However, it is unclear which subset of this patient population will respond to specific chemotherapies and which will not.

The collagen gel droplet-embedded culture drug sensitivity test (CD-DST) is an in vitro anticancer drug sensitivity test [3–7] that can be performed using resected tumor samples. Although George et al. reported that most patients with synchronous stage IV CRC who receive chemotherapy never require palliative surgery for the primary tumor [8], some patients undergo primary tumor resection in response to various complications (e.g., bleeding, perforation, obstruction).

Recent studies have reported that CD-DST can provide valuable therapeutic information in patients with gastric cancer, lung cancer, colorectal cancer, and pancreatic cancer [9–13]. Thus, the goal of the present study was to evaluate outcomes of patients with stage IV CRC who received chemotherapy based on the results of CD-DST.

Patients and methods

Patients

We investigated 38 patients with stage IV CRC who underwent treatment between November 2005 and April

2011 at Shiga University of Medical Science in Japan. All patients were younger than 85 years and had untreated evaluable metastatic lesions that were diagnosed by computed tomography (CT), 18F-fluorodeoxyglucose positron emission tomography-CT (FDG-PET-CT), and/or diffusion-weighted magnetic resonance imaging (DW-MRI). The primary tumors were surgically resected, and all tissue samples were investigated by CD-DST to evaluate their chemosensitivities. All samples were histologically confirmed as colorectal adenocarcinoma. Patients treated with in vitro sensitive drugs were defined as Group A ($n = 14$), and patients treated with in vitro non-sensitive drugs were defined as Group B ($n = 24$). Written informed consent was obtained from each patient prior to chemotherapy.

Collagen gel droplet-embedded culture drug sensitivity test (CD-DST)

The CD-DST was performed using tumor tissue as described by Kobayashi [6] and Kobayashi et al. [7]. Briefly, surgically resected specimens were digested in dispersion collagenase enzyme, and the dispersed cancer cells were incubated in a collagen gel-coated flask. Then, the viable cells adhering to the collagen gel layer were collected and were added to reconstructed Type 1 collagen solution (Cell matrix Type CD^{TM}, Kurabo, Osaka, Japan). Three drops of these mixtures were placed in each well of a 6-well plate, and then 5-fluorouracil (5-FU) (1.0 μg/ml), irinotecan (SN38) (0.03 μg/ml), oxaliplatin (OHP) (0.5 μg/ml), 5-FU/SN38 (1.0, 0.03 μg/ml), or 5-FU/OHP (1.0, 0.5 μg/ml) were added to each well. Plates were incubated for 24 h. After removal of the medium containing anticancer drug, each well was incubated with PCM-2 medium (Kurabo) for 7 days. The in vitro chemosensitivity effect of each agent was expressed as a ratio of the total colony volume (T) of the treated cells to that of the untreated cells (C). In our study, a sample with a ratio of T to C of 60 % or less was regarded as sensitive [8].

Assessments

Histories, physical examinations, laboratory tests, and safety assessment were performed pretreatment and weekly thereafter. Carcinoembryonic antigen (CEA) and CA19-9 were measured at least every 4 weeks. Chemotherapy dose adjustments were determined on an individual basis. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria (version 3.0). The initial chemotherapy regimen was maintained for at least two cycles. This treatment regimen was discontinued upon tumor progression, grade 3 or 4 toxicity, or at the patient's request. Patients underwent close follow-up with diagnostic imaging after their first chemotherapy. CEA levels, abdominal CT, and chest CT were checked every 2 months. Secondary or tertiary chemotherapy was administered on an individual basis.

Responses were evaluated after 2 months from initial administration using the response evaluation criteria in solid tumors (RECIST).

Statistical analysis

The primary outcome was overall survival, and secondary outcomes were progression-free survival and tumor response. Overall survival was calculated from the date of initial surgery until the date of death. Progression-free survival was measured from the date of initial surgery until the date of disease progression or death. Patients who did not have disease progression and patients who died were excluded at the date of their last follow-up. Overall survival and progression-free survival were analyzed with the use of Kaplan–Meier curves, and differences between the curves were tested with the generalized Wilcoxon test. We conducted analyses using Excel (Microsoft, Redmond, WA) and Statcel2 (OMS Publisher, Saitama, Japan) software. A p value of <0.05 was considered statistically significant.

The Student's t test and χ^2 test were used to compare data. A p value of <0.05 was considered statistically significant.

Results

Patient characteristics and CD-DST

Patient characteristics are shown in Table 1. Baseline characteristics were balanced between the two groups. In Group A, primary tumors were in the colon in seven patients and in the rectum in seven patients, In Group B, primary tumors were in the colon in 10 patients and in the rectum in 14 patients. All patients in both groups had an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1.

Results of CD-DST are shown in Table 2. Median T/C ratio in Group A was lower than that of Group B. The drug sensitivity of Group A tended to be higher than that of Group B.

Outcomes

Site of metastasis, first chemotherapy, administration of molecularly targeted drug, response rate, and metastatic lesion resection in Group A and Group B are shown in Table 3. The metastatic site in Group A was the liver in 11 patients, peritoneum in two patients, lung in three patients,

Table 1 The characteristics of patients with metastatic colorectal cancer

	Group A ($n = 14$)	Group B ($n = 24$)	p value
Age (median, years)	65.64 (52–85)	65.16 (36–81)	0.449
Gender (male/female)	7/7	17/7	0.199
Primary tumor site colon/rectum	7/7	10/14	0.61
ECOG performance status (0/1/2/3/4)	13/1/0/0/0	21/3/0/0/0	

Eastern Cooperative Oncology Group (ECOG)

Table 2 The results of CD-DST in Group A and Group B

	T/C (%) in Group A	T/C (%) in Group B
5-FU	71.83 (43–86.28)	77.69 (45.3–100)
SN38	65.54 (0–100)	67.05 (32.15–100)
OHP	66.9 (43.9–100)	74.68 (58.1–100)
5FU/SN38	62.4 (0–100)	66.0 (40.5–100)
5FU/OHP	61.0 (40.2–100)	70.7 (0–100)

The CD-DST method was employed to study in vitro growth inhibition, as previously described. The in vitro sensitivity was expressed as the T/C ratio, in which T is the total volume of living cancer cells in the treated group and C is the total volume of living cancer cells in the control group. Positive, T/C <60 %; negative, T/C ≥60 %

5-FU 5-fluorouracil, *SN38* the active metabolite of irinotecan, *OHP* the active metabolite of oxaliplatin

and bone in one patient. The metastatic site in Group B was the liver in 17 patients, lymph node in two patients, peritoneum in two patients, lung in 10 patients, and bone in one patient. There were three patients with two or more sites of metastasis in Group A. There were five patients with two or more sites of metastasis in Group B. The initial chemotherapeutic regimen in Group A was 5-fluorouracil/leucovorin/oxaliplatin (FOLFOX) in five patients, 5-fluorouracil/leucovorin/irinotecan (FOLFIRI) in six patients, and other regimens in three patients. The initial chemotherapeutic regimen in Group B was FOLFOX in 19 patients, FOLFIRI in two patients, and other regimens in three patients. A molecularly targeted drug was used for four patients in Group A and for nine patients in Group B.

Among the 14 patients in Group A, there was one complete response (CR), 11 partial responses (PR), two stable diseases (SD), and zero progressive diseases (PD). The response rate in Group A was 85.71 %. Among the 24 patients in Group B, there were one CR, nine PR, 10 SD, and four PD, respectively. The response rate in Group B was 41.67 %. The response rate of Group A was significantly higher than that of Group B; $p = 0.0079$. Table 4 shows the relationship between patient treatments and CD-DST results for 5-FU/SN38 versus 5-FU/OHP. A T/C ratio of 5-FU/OHP was significantly lower than that of 5-FU/SN38 in sensitive patients with FOLFOX. A T/C ratio of 5-FU/SN38 was significantly lower than that of 5-FU/OHP in sensitive patients with FOLFIRI. The median PFS was 696.5 days in Group A and 297.5 days in Group B ($p = 0.0326$; Fig. 1). The median OS was 1,023.4 days in Group A and 518.5 days in Group B ($p = 0.0061$, Fig. 2).

Table 3 Summarized data of the patients

	Group A ($n = 14$)	Group B ($n = 24$)	p value
Sites of metastases			
Liver	11	17	
Lymph node	0	2	
Peritoneum	2	2	
Lung	3	10	
Bone	1	1	
2 or more sites	3	5	
Prior chemotherapy			
FOLFOX	5	19	
FOLFIRI	6	2	
Other	3	3	
Molecular target drug			
Yes	4	9	
No	10	15	0.57
Response rate (%)	85.71	41.67	
CR	1	1	0.0079
PR	11	9	
SD	2	10	
PD	0	4	
Metastasectomy			
Liver	4	2	
Lung	0	1	
Lymph node	0	0	0.076
Dissemination	2	1	
Other	0	0	
2 or more lesion	0	0	

FOLFOX 5-fluorouracil/leucovorin/oxaliplatin, *FOLFIRI* 5-fluorouracil/leucovorin/irinotecan, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

Discussion

The present study demonstrated that CD-DST is useful to identify effective anticancer drugs for individual patients with stage IV CRC; patients treated with chemotherapy that was consistent with the tumor CD-DST profile achieved more favorable responses when compared with patients whose tumors were shown to be relatively resistant to chemotherapy by this test. This is the first report to

Table 4 The relationship between patient treatments and the results of CD-DST

	T / C (%) 5-FU / SN38	T / C (%) 5-FU / OHP
Sensitive patints with FOLFOX (n=11)	85.9 (58-100) (n=11)	67.4 (40.2-100) (n=11)
	p=0.015	
Sensitive patints with FOLFIRI (n=6)	45.2(0-100) (n=6)	79.1 (58-100) (n=6)
	p=0.009	
Resistant patints with FOLFOX (n=13)	95.6 (77-100) (n=13)	95.7 (75-100) (n=13)
	p =0.397	
Resistant patints with FOLFIRI (n=2)	54.0 (49-59) (n=2)	62.5 (50-75) (n=2)
	p=0.356	

The patients achieved CR or PR by first chemotherapy were defined as sensitive patients, and the patients achieved SD or PD by first chemotherapy were defined as resistant patients. FOLFOX, 5-fluorouracil/leucovorin/oxaliplatin; FOLFIRI, 5-fluorouracil/leucovorin/irinotecan

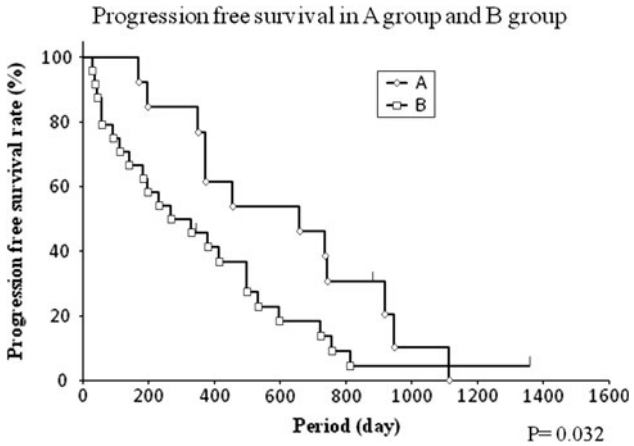

Fig. 1 Kaplan–Meier curves for progression-free survival (PFS). Median PFS was 696.5 days in Group A and was 297.5 days in Group B

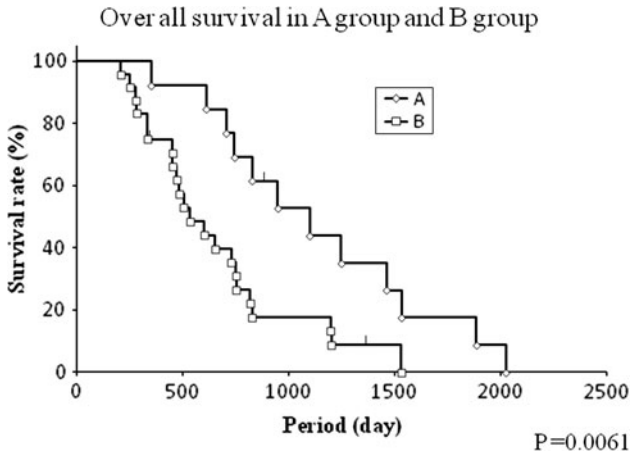

Fig. 2 Kaplan–Meier curves for overall survival (OS). Median OS was 1,023.4 days in Group A and was 518.5 days in Group B

demonstrate the clinical potential of CD-DST in patients with stage IV CRC.

Stage IV CRC is a lethal disease [1], and tumor resection in these patients is of uncertain benefit. George et al. reported that 93 % patients with synchronous stage IV CRC who received chemotherapy never require palliative surgery for the primary tumor [9]. Of 233 patients, 16 patients (7 %) required emergent surgery for major complications (e.g., bleeding, perforation, obstruction) that involved the primary tumor [9]. Primary tumor resection is performed in presence of symptoms. Thus, primary tumor resection should be considered on an individual basis. One potential benefit to tumor resection is highlighted by the improved outcomes in response to chemotherapy that was consistent with the tumor CD-DST profile in this study.

Management of stage IV CRC may consist of medical treatments (conventional systemic chemotherapy, molecularly targeted agents) and/or surgery. While 5-FU, irinotecan, and oxaliplatin are the standards of care for the treatment of colorectal cancer, some patients may be resistant to these therapies. Therefore, we performed CD-DST after initial surgery to identify what would theoretically be the most appropriate anticancer regimen.

The CD-DST is an in vitro anticancer drug sensitivity test [3–8]. One of the advantages of CD-DST, when compared with previous anticancer drug sensitivity tests, is that it uses a three-dimensional growth assay with an image analysis device that can differentiate cancer cells from fibroblast cells [6]. Indeed, recent studies have reported that CD-DST can provide useful therapeutic information in patients with gastric cancer, lung cancer, colorectal cancer, or pancreatic cancer [10–14]. Furthermore, CD-DST can assess sensitivity to relatively newer agents, such as 5-FU, oxaliplatin, and irinotecan as well as to combination therapy with 5-FU/SN38 and 5-FU/OHP.

The present study demonstrated a good correlation between in vitro drug sensitivity and patient outcomes; patients treated with regimens that were deemed "sensitive" according the CD-DST assay had better outcomes (better response and longer PFS) when compared with those patients who were treated with regimens that were deemed "insensitive." Other treatment such as metastasectomy and coagulation therapy may be indicated if better response is achieved. These additional treatments may yield better outcomes [15–19]. On the other hand, our data showed better OS in patients treated with in vitro sensitivity-based chemotherapy and worse survival in the patients treated with in vitro non-sensitive drugs. The patients in Group B had worse responses when compared with Group A. PFS and OS in Group B was significantly worse than Group A, while the response rate of Group B was 41.67 %. The CD-DST defined chemosensitive and chemoresistant tumors, and patients with tumors that are insensitive to conventional chemotherapies should be considered for alternative treatment strategies, such as surgery and molecularly targeted drugs [15–17, 20–22]. This would have the benefit of avoiding the side effects associated with systemic therapies in patients who would not otherwise benefit from such therapy [20–22]. In fact, the use of CD-DST might be one of the tools to supplement informed consent prior to initiation of therapy.

In summary, the CD-DST can define chemoresistant and chemosensitive tumors. The present results support the development of a randomized trial between chemotherapy predicted by CD-DST to be most active versus a single defined regimen used as standard therapy in those cases where the CD-DST cannot define a most active regimen.

Conflict of interest The authors declare that they have no conflict of interest.

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