A Clinical Potential of the Anti-Cancer Drug Sensitivity Test for Patients with Endocrine Cell Carcinoma of the Rectum: Report of a Case

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Abstract
Endocrine cell carcinoma (ECC) of the rectum is a rare neoplasm usually associated with a poor prognosis. No standard chemotherapeutic regimens exist for the treatment of ECC. We encountered a case of rectal ECC in which chemotherapy was administered based on the results of a chemosensitivity test, the collagen gel droplet-embedded culture drug sensitivity test (CD-DST). A 64-year-old man with anal pain was diagnosed with rectal cancer. The patient received Mile’s operation and was microscopically diagnosed as having ECC with foci of differentiation to adenocarcinoma. Multiple hepatic metastases were found 1 month after surgery. The patient received TEGAFIRI (oral UFT and leucovorin with irinotecan), which was intermediated sensitive based on the CD-DST. TEGAFIRI initially reduced the extent of hepatic metastases, indicating a partial response after 3 months of administration. However, progression of the disease was confirmed and TEGAFIRI had become ineffective after 5 months of administration. Thereafter, although mFOLFOX6 with bevacizumab, which was insensitive based on the CD-DST, was administered, the progression of hepatic metastases continued, indicating progressive disease. The patient died 13 months after surgery. In conclusion, we suggest that CD-DST may be useful for identifying sensitive antitumor agents for the treatment of rectal ECC for which no standard chemotherapeutic regimens exist.

Key words: rectal cancer, chemotherapy, CD-DST

Introduction
Endocrine cell carcinoma (ECC) of the rectum is a rare neoplasm that is usually associated with a poor prognosis. Almost all patients have metastases in the liver and lymph nodes at the time of diagnosis. Radical surgery with lymph node dissection is performed when possible. No standard chemotherapy regimen exists to treat this tumor type¹.

The collagen gel droplet-embedded culture drug sensitivity test (CD-DST) is a new in vitro anticancer drug sensitivity test⁷. Recent studies have reported that CD-DST has the potential to provide therapeutic information in patients with gastric cancer⁹, lung cancer⁹, colorectal cancer⁹ and pancreatic cancer⁹.

We encountered a rare case of rectal ECC. CD-DST revealed that the resected tumor was intermediately sensitive to a antitumor drug. We were able to perform chemotherapy based on the CD-DST results in this case. To the best of our knowledge, this is the first report to demonstrate the clinical potential of CD-DST in a patient with rectal ECC.

Case Report
A 64-year-old male presented with anal pain. A
3-cm tumor was detected on the left side of the lower rectum on digital rectal examination. Barium enema and endoscopy revealed a submucosal tumor above the dentate line of the rectum (Fig.1A, B). Abdominal computed tomography (CT) and Magnetic resonance image (MRI) revealed a 3-cm tumor in the left side of the rectum that primarily extended outside of the rectal wall (Fig.1C-E). Biopsy of the rectal tumor suggested a poorly differentiated adenocarcinoma. Laboratory analysis revealed a carcinoembryonic antigen (CEA) level of 220.8 ng/mL and a CA19-9 level of 104 ng/mL. The patient underwent rectal resection with lymph node dissection (Miles’ operation), after which patient’s staging was determined to be T3, N2, M0, Stage III (Fig.2A). Microscopically, the surgical specimen was diagnosed as ECC with foci of differentiation to adenocarcinoma (Fig.2B). Immunohistochemical staining revealed that expression of the neuroendocrine markers chromogranin, CD56 (NCAM), and synaptophysin was positive; these results were compatible with ECC (data not shown). The tumor invaded into the skeletal muscle; however, the resected margin and surface were clear of cancer cells. Three regional lymph node metastases along with the primary feeding artery of the tumor were identified. CD-DST revealed that the tumor was sensitive to certain chemotherapeutic agents, as shown in Table 1. Hepatic metastases were found 1 month after initial surgery (Fig.3A). At this point, the tumor markers had decreased somewhat, but still remained high (CEA level, 40.3 ng/mL; CA19-9 level, 40 ng/mL).

Since the CD-DST results indicated that the tumor was most sensitive to 5-FU/SN38, which simulates the FOLFIRI combination, the patient underwent TEGAFIRI chemotherapy. The patient received oral UFT 600 mg/day and leucovorin 75
Fig. 2  Gross and microscopic appearance of the resected tumor.
A: The tumor primarily extended outside of the rectal wall. A small ulcer was found above the dentate line.
B: ECC with foci of differentiation to adenocarcinoma was observed. Mitotic figures were frequently identified. Marked lymph and venous invasion was observed upon hematoxylin and eosin (HE) staining.

mg/day combined with irinotecan 200 mg/m² every 2 weeks. The size of the hepatic metastases by CT scan reduced approximately 41% without development of new lesions (Fig.3B) and tumor marker levels (CEA level, 14.8 ng/mL; CA19-9 level, 28 ng/mL) had decreased 3 months after TEGAFIRI administration; this was characterized as stable disease (SD) according to the Response Evaluation Criteria In Solid Tumors (RECIST). Although we did not follow the tumor size by CT every month, we confirmed that the size of hepatic metastasis reduced more than 20% at 2 months after the initiation of FEGAFIRI by ultrasonography. At this time point, the levels of tumor markers were lowest during the treatment period (CEA level, 128 ng/mL; CA19-9 level, 34 ng/mL). Therefore, we believe that this first response induced by TEGAFIRI was partial response (PR). However, at 5 months after TEGAFIRI administration, both the size of the hepatic metastases (approximately 320%; Fig.3C) and tumor marker levels (CEA level, 55.0 ng/mL; CA19-9 level, 45.0 ng/mL) increased, indicative of progressive disease (PD) and demonstrating that TEGAFIRI had become ineffective. Thereafter, mFOLFOX6 with bevacizumab, the second best regimen, was administered, although it is insensitive according to the result of CD-DST. Tumor marker levels gradually increased every month with this new regimen. New metastatic lesions in the liver (Fig.3D) were confirmed 3 months after initiation of mFOLFOX6 with bevacizumab by CT scan, again indicating PD. Unfortunately, the hepatic metastases continued to progress, and the patient died 13 months after surgery (Fig.3).

Discussion
The incidence of ECC in the rectum is reported to represent 0.1-3.9% of colorectal cancer cases. In the majority of cases (69.4%), metastatic tumors were found in distant organs at the time of rectal ECC diagnosis[6]. These tumors tend to rapidly progress and are therefore associated with a very poor prognosis. ECCs of the rectum comprise approximately 10% of all gastrointestinal ECCs. Most appear during endoscopy as small (<1 cm), movable submucosal tumors. Metastases only tend to occur in association with tumors that are ≥2 cm and that invade the muscularis propria[7]. Berneck et al. reported that for 38 cases of colorectal ECC, the mean survival period was 10.4 months, and 3-year survival was 13%[8]. The recommended treatment for rectal ECC is generally intestinal resection with lymph node dissection in resectable cases; however, no standard chemotherapy regimen has been established for treatment of this tumor type.

Since rectal ECC is generally considered to be biologically similar to small cell carcinoma of the lung, almost all previously used chemotherapeutic regimens were based on pulmonary small cell carcinoma regimens. Redman et al. demonstrated that a regimen containing cyclophosphamide, doxorubicin, and vincristine reduced the size of liver metastases of rectal ECC[9]. Schwartz et al. reported the efficacy of a systemic chemotherapeutic regimen containing etoposide and cisplatin[10]. In Japan, since Okuyama et al. demonstrated that combined 5-FU and cisplat-
CD-DST & rectal endocrine cell carcinoma

Table 1  Summary of antitumor drug sensitivity test results (CD-DST)

<table>
<thead>
<tr>
<th>Agent</th>
<th>T/C (%)</th>
<th>Agent</th>
<th>T/C (%)</th>
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<tbody>
<tr>
<td>5-FU</td>
<td>86.28</td>
<td>SN38</td>
<td>70.25</td>
</tr>
<tr>
<td>mitomycin C</td>
<td>u. d.</td>
<td>oxaliplatin</td>
<td>u. d.</td>
</tr>
<tr>
<td>gemcitabine</td>
<td>83.60</td>
<td>vinorelbine</td>
<td>80.61</td>
</tr>
<tr>
<td>docetaxel</td>
<td>100.00</td>
<td>5-FU/SN38</td>
<td>58.35</td>
</tr>
<tr>
<td>epirubicin</td>
<td>u. d.</td>
<td>5-FU/oxaliplatin</td>
<td>67.66</td>
</tr>
<tr>
<td>cisplatin</td>
<td>u. d.</td>
<td></td>
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The CD-DST method employed to study in vitro growth inhibition has been documented previously. 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. T/C<50%: Sensitive, T/C=50%-60%: intermediate sensitive, T/C>60%: insensitive. 5-FU, 5-fluouracil; SN38, the active metabolite of irinotecan; u. d., undetectable due to a limited number of cells.

Fig. 3 Clinical course.
The metastatic tumor is marked by a single arrow. The new lesion was detected in segment 2 at 9 months after surgery (double arrows). TEGAFIRI: Irinotecan 200 mg/m² days 1 and 15; UFT 600 mg/day and leucovorin 75 mg/day on days 1-5 and stopped on days 6 and 7. mFOLFOX6 + bevacizumab: Oxaliplatin 85 mg/m², Leucovorin 200 mg/m², 5-FU 400 mg/m² (bolus), 5-FU 2,400 mg/m² (46 hours continuous) + bevacizumab 10 mg/kg.

in dramatically diminished hepatic metastases and improved patient quality of life, the 5-FU and cisplatin combination is commonly used in Japanese case reports. Recently, FOLFOX and FOLFIRI have become standard chemotherapeutic regimens for the treatment of colorectal cancer. Therefore, a few recent case reports have used irinotecan or oxaliplatin-containing regimens for treatment of rectal ECC. Regimens containing irinotecan or oxaliplatin for the treatment of ECC in other organs, such as the breast and pancreas, have been recently reported; however, to the best of our knowledge, only a
few reports have demonstrated the clinical efficacy of irinotecan- or oxaliplatin-containing regimens in patients with ECC of the rectum. Thus, present case also suggest that FOLFIRI may also be effective regimens in the treatment of rectal ECC.

CD-DST cultures extract cancer cells three-dimensionally in a collagen gel droplet. Three-dimensional culture with collagen matrix is preferable to establish cell culture from human cancer tissue. This characteristic has made it possible to measure chemosensitivity with as little as $1 \times 10^5$ cancer cells, which are typically present in one or two specimens biopsied by bronchoscope[13]. Herein we have reported that CD-DST may provide useful information for enabling chemotherapy regimens to be tailored against the tumors of individual patients[7, 8].

The sensitivity of CD-DST 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. Usually, a T/C ratio of 50%[16] to 60%[17] or less is determined to have in vitro sensitivity in CD-DST. The relationship between the T/C ratio of CD-DST and clinical response has been previously reported. Kawamura et al. demonstrated a good correlation between in vitro drug sensitivity and clinical response in patients with unresectable non-small cell lung cancer, with a true positive rate of 72.7% and a true negative rate of 100%; the sensitivity, specificity, and accuracy were 100%, 64.7%, and 81.8%, respectively[16]. Higashiyama et al. reported that in malignant pleural mesothelioma patients, the correlation between CD-DST results and clinical response (including PR) had 100% sensitivity, 36% specificity, a 30% positive predictive value, a 100% negative predictive value, and 50% accuracy[17].

According to our recent data, T/C of 60% may differentiated clinical response of adjuvant chemotherapy following Stage II and III colorectal cancer (unpublished data). Therefore, we indicated 50% - 60% of T/C to consider having immediately sensitive response. Since T/C rate in this case was less than 60% according to the result of 5-FU/SN38 combination in CD-DST, the clinical observation of PR induced by TEGAFIRI was reasonable based upon the idea of cut-off value of 60%T/C. However, the second best regimen whose T/C was >60%, mFOLF-OX6 with bevacizumab, resulted in PD within 3 months. These clinical responses were also compatible with the CD-DST results.

In conclusion, tailored chemotherapy developed using CD-DST may be useful for patient with rare cancer for which no standard chemotherapeutic regimens exist. We suggested that CD-DST might provide beneficial information about the antitumor agents that may be effective for treating rectal ECC in individual patients.

References
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