Direct evidence that heterogeneity necessitates and limits the use of multidrug chemotherapy in colon cancer

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Abstract. Considerable progress in the form of multidrug chemotherapy has recently been made in chemotherapy for the prolongation of survival in advanced colon cancer. It is generally accepted that colon cancer is biologically heterogeneous for multiple properties, including sensitivity to chemotherapeutic agents and metastasis. Although this partly explains the success of multidrug chemotherapy, there has been no direct evidence that multidrug regimens affect individual heterogenous cancer characteristics in colon cancer. Here, we present a case of metachronous ovarian metastasis in a colon cancer patient with dissemination who underwent irinotecan-based followed by oxaliplatin-based chemotherapy. We were able to obtain three samples from the patient, one of primary cancer and two of metastatic tumors from secondary surgery. Of note, both chemoresistant and chemosensitive tumors were present in the patient at the same time. To understand the influence of multidrugs on individual cancer characteristics, we examined differences in the molecular characteristics of the three samples using RT-PCR, focusing in particular on alterations in chemoresistant genes. In shrunken peritoneal metastasis, we found a significant increase in the mRNA levels of an irinotecan-sensitive gene, although other molecular factors were resistant to both 5-FU and oxaliplatin. We also confirmed that the recurrent ovarian tumor showed significant resistance to all three drugs: 5-FU, irinotecan and oxaliplatin. These results suggest that the heterogeneity of colon cancer necessitates and limits the use of multidrug chemotherapy.

Introduction

Over the past few decades, considerable progress in chemotherapy for advanced colon cancer has been made in terms of the prolongation of survival. The integration of oxaliplatin and irinotecan as conventional cytotoxic agents, as well as the use in standard medical therapy of bevacizumab and the epidermal growth factor receptor antibody cetuximab as novel targeted agents, have turned metastatic colon cancer into a disease which, for most patients, now has an expected overall survival of more than two years - over twice as long as it was about ten years ago (1-6).

An important recent finding in colon cancer chemotherapy has been the association of improved overall survival with the use of three effective chemotherapy agents (5-FU, irinotecan and oxaliplatin) at some point during the course of treatment (7). In addition, current data support the use of multidrug regimens, whether as first or second-line treatment, rather than sequential single-agent therapy (8). However, the significance of combination therapy has not yet been determined in detail, despite its resounding clinical success.

It is generally accepted that colon cancer develops due to genetic alterations, and that cancers are biologically heterogeneous for multiple properties, including antigenicity, sensitivity to chemotherapeutic agents, invasion and metastasis (9,10). Although this may partly explain the success of multi-target therapeutic strategies in combination with chemotherapy, there has been no direct evidence that multidrug regimens affect individual heterogenous cancer characteristics in colon cancer.

This report presents a case of metachronous ovarian metastasis in a colon cancer patient with dissemination who underwent irinotecan-based followed by oxaliplatin-based chemotherapy. We were able to obtain three samples from the patient, one of primary cancer and two, from secondary surgery, of chemoresistant tumor (ovarian metastasis) and of chemosensitive tumor (peritoneal metastasis). The focus of the study was on whether the expression of chemoresistant genes is altered in primary compared to secondary resection samples under multidrug chemotherapy. The significance of multidrug regimens in metastatic colon cancer treatment is also discussed.

Case report

In 2003, a 70-year-old woman was diagnosed with ascending colon cancer and underwent a right-side hemicolectomy. Histopathological examination revealed the malignancy to be mucinous adenocarcinoma. No lymph node metastases were found in the resected specimen. The post-operative course was

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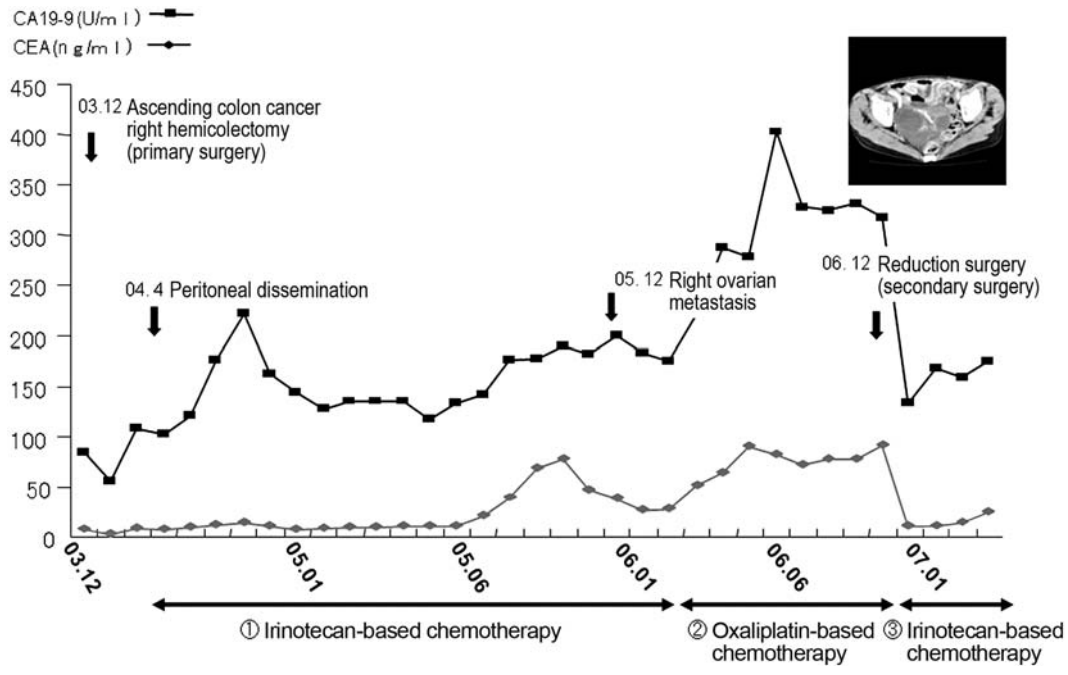

Figure 1. Clinical course and changes in the patient's serum CEA and CA19-9 levels.

uneventful, and adjuvant chemotherapy was not performed. During a post-surgery routine, serum tumor markers such as carcinoembryonic antigen (CEA) and CA19-9, which were initially normalized by the operation, were found to be suddenly increased. Although the patient remained asymptomatic and computed tomography (CT) was not remarkable, serum tumor markers progressively increased. F-18 fluorodeoxyglucose positron emission tomography (FDG-PET) was performed to evaluate occult recurrence, and revealed multiple hot spots in the abdomen and pelvis. Four months after the operation, the patient was diagnosed with peritoneal dissemination from ascending colon cancer.

The patient was initially treated with a combination of fluoropyrimidine and irinotecan. This regimen was well received, and was subsequently repeated each week. Though the disease was stabilized for approximately two years, a CT scan twenty months after the introduction of initial chemotherapy revealed a metastatic right ovarian tumor, and the chemotherapy regimen was changed to FOLFOX 6. Other abnormalities were not found in the abdominal cavity. However, increases in the serum CEA and CA19-9 levels did not cease after the initiation of FOLFOX 6 treatment, and the metastatic right ovarian tumor gradually increased in size. Therefore, the patient underwent cytoreductive surgery thirty-two months after the introduction of initial chemotherapy (Fig. 1).

Laparotomy revealed, in addition to extensive ovarian metastasis, cicatrized peritoneal metastasis that exhibited the effects of treatment in the pelvic cavity. The patient underwent bilateral oophorectomy and a reduction of the residual peritoneal metastasis. No macroscopic residual metastases remained in the intra-abdominal cavity and, at histological review, the chemosensitive peritoneal metastasis displayed pronounced tumor necrosis and lytic change, while the chemoresistant tumor exhibited no pathological effects in response to chemotherapy (Fig. 2). The patient now had a carcinomatous peritonitis; however, chemotherapy was undertaken at an outpatient clinic, and the patient is still alive over forty-six months after initial treatment.

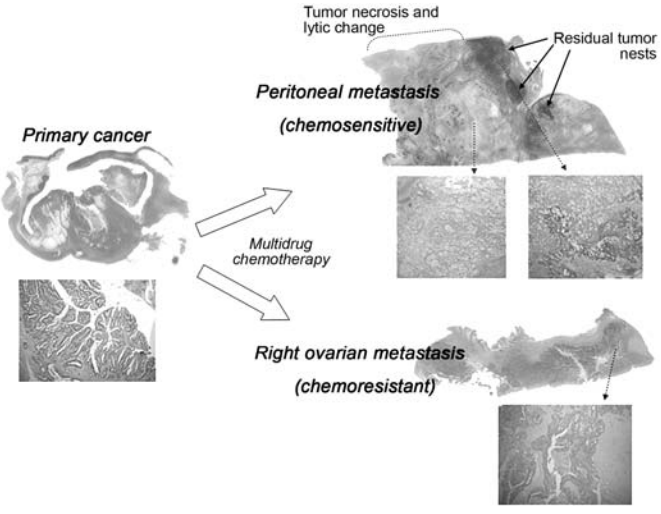

Figure 2. Chemosensitive peritoneal metastasis displayed pronounced tumor necrosis and lytic change, while chemoresistant ovarian metastasis exhibited no pathological effects in response to chemotherapy.

Materials and methods

Patient samples. The study was conducted at the Department of Gastrointestinal and Pediatric Surgery, Division of Reparative Medicine, Institute of Life Sciences, Mie University Graduate School of Medicine. Local ethics committee approval was obtained, as well as appropriate informed written consent from the patient. Fresh surgical specimens of the primary ascending colon cancer and of two different metastases from secondary surgery, right ovarian metastasis and pelvic peritoneal metastasis, were harvested under sterile conditions.

Table I. Primer sets used for reverse-transcription polymerase chain reaction.

Gene		Primers
TS	Sense	5'-GCCTCGGTGTGCCTTTCA-3'
	Antisense	5'-CCCGTGATGTGCGCAAT-3'
DPD	Sense	5'-AGGACGCAAGGAGGGTTTG-3'
	Antisense	5'-GTCCGCCGAGTCCTTACTGA-3'
TP	Sense	5'-CCTGCGGACGGAATCCT-3'
	Antisense	5'-TCCACGAGTTTCTTACTGAGAATGG-3'
OPRT	Sense	5'-CCAGGAGTTCAGTTGGAAGC-3'
	Antisense	5'-GGAACCTCGTTTGCCAATAA-3'
ERCC1	Sense	5'-GGGAATTTGGCGACGTAATTC-3'
	Antisense	5'-GCGGAGGCTGAGGAACAG-3'
Top1	Sense	5'-ACAACGATTCCCAGATCGAA-3'
	Antisense	5'-CGGTGTTCTCGATCTTTGTG-3'
CE2	Sense	5'-AGTGGTGTGAGGGATGGAAC-3'
	Antisense	5'-TGGCTAAGAAACTCTGACTCCA-3'
ß-actin	Sense	5'-ACAGAGCCTCGCCTTTGC-3'
	Antisense	5'-GCGGCGATATCATCATCC-3'

These specimens were immediately placed in liquid nitrogen and stored at -80˚C until use. A histopathological examination was performed on the 10% formalin-fixed, paraffin-embedded specimens by a pathologist from the university's pathology division. Use of patient material was in accordance with Institutional Review Board guidelines and protocol.

Reverse-transcription PCR analysis. Total-RNA from primary colon cancer and two metastases was extracted using an RNeasy Mid Kit (Qiagen Inc., Chatsworth, CA) according to the manufacturer's instructions. Reverse-transcription polymerase chain reaction (RT-PCR) was performed using the specific primers listed in Table I. Optimum cycling parameters in the linear phase of amplification consisted of 23-28 cycles of 30 sec denaturation at 94˚C, 30 sec annealing at 60˚C and 1 min elongation at 72˚C for selected genes. Control PCR (25 cycles) was also performed with ß-actin as a standard for sample normalization. Amplified products were electrophoretically separated, vizualized and photographed under UV light after ethidium bromide staining, then quantified using the CS Analyzer version 2.0 (Atto, Tokyo, Japan).

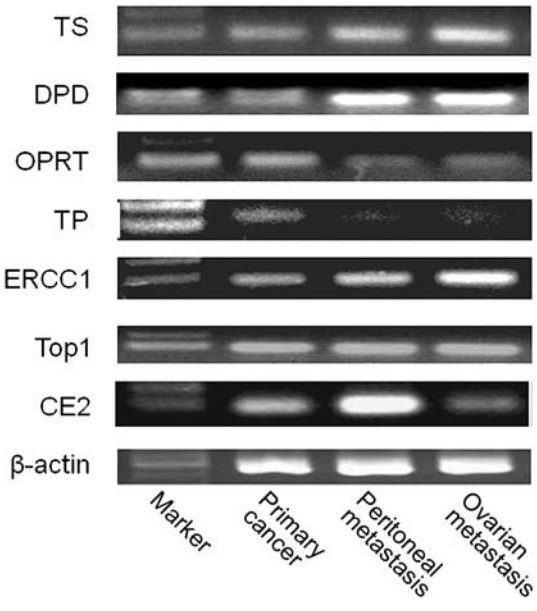

Figure 3. Expression patterns of drug sensitivity genes in the primary and metastatic lesion: TS, DPD, TP,OPRT,CE2, Top1 and ERCC1.

Results

Semi-quantitative reverse-transcription PCR analysis was used to analyze the expression levels of a number of genes that have been implicated in the determination of sensitivity to 5-FU, oxaliplatin and irinotecan-based chemotherapy. In the chemoresistant right ovarian tumor, both the 5-FU target enzyme thymidylate synthase (TS) and the 5-FU-catabolizing enzyme dihydropyrimidine dehydrogenase (DPD) were over-expressed compared to the primary site. Expression of the 5-FU-anabolizing enzyme thymidine phosphorylase (TP) and orotate phosphoribosyl transfenase (OPRT) was lower in the ovarian metastasis compared to primary site. Carboxylesterase2 (CE2) mRNA expression was lower in the ovarian than in the primary site, although expression of topoisomerase I (Top I) mRNA, a target of irinotecan, did not differ between the lesions. Significant increases in the mRNA levels of excision repair cross-complementation group1 (ERCC1) were also found when compared with the primary cancer (Fig. 3). These

results indicate that the ovarian metastasis was resistant to all three (5-FU, irinotecan and oxaliplatin) effective agents following multidrug chemotherapy with irinotecan and oxaliplatin-based chemotherapy.

In the chemosensitive peritoneal metastasis, significant increases in the mRNA levels of CE2 as compared to the primary cancer were found, although expression of Top I mRNA did not differ between the lesions. The same alterations in 5-FU related genes (TS, DPD, TP, OPRT) and in the oxaliplatin related gene (ERCC1) were detected as had been seen in the ovarian metastasis. These results indicate that the peritoneal metastasis was sensitive to irinotecan-based chemotherapy not only clinically but also basically.

Discussion

The principle behind approaches to medical therapy in oncology is the notion that, once tumors show signs of progression under a certain chemotherapy regimen, subsequent lines of treatment should be altered and based on non-cross-resistant agents. However, clinical experience in colon cancer clearly demonstrates that the same agents can be effectively reused in later phases of therapy. In other words, the addition, not change, of chemotherapeutic agents is characteristic in chemotherapy regimens for colon cancer. Although some of the efficacy of these reused agents comes from the use of distinct synergistic mechanisms with other agents, the basic significance of a multidrug regimen in colon cancer has yet to be explained.

In addition, there remains a clinical problem in the treatment of cancer. Despite the drastic improvements in survival rates afforded by chemotherapy, almost all colon cancer patients eventually die of the disease due to the acquisition of drug resistance. Drug resistance is therefore a major concern that limits the effectiveness of chemotherapy used to treat cancer. Tumors may be intrinsically resistant to chemotherapy prior to treatment, or may acquire resistance during the course of chemotherapy, even when they were initially sensitive to the treatment. Whether intrinsic or acquired, drug resistance is believed to be the reason for treatment failure in almost all patients with metastatic cancer. Clearly, if drug resistance could be overcome, the impact on survival would be highly significant.

In the present case, three samples from one female patient who had undergone irinotecan-based followed by oxaliplatin-based chemotherapy were obtained: one primary cancer and two metastatic tumors from secondary surgery. Interestingly, the chemoresistant and chemosensitive tumors were found to exist concurrently in the patient. To understand the influence of multidrugs on individual cancer characteristics, we examined differences in the molecular characteristics of the three samples, focusing in particular on alterations in chemoresistant genes.

The mechanisms involved in resistance to chemotherapy usually entail the up-regulation of resistance mechanisms or the down-regulation of the target and its related genes. Examples of the former include repairing DNA damage such as ERCC1, known as l-OHP resistant factor (11,12), while the latter include topoisomelase I (Top 1), irinotecan and CE targets, the active metabolite to the target (13,14). As for 5-FU, its target is thymidylate synthase (TS) and its representative rate-limiting enzymes are dihydropyrimidine dehydrogenase (DPD), thymidine phosphorylase (TP) and orotate phosphoribosyl transfenase (OPRT) (15-18). To best clarify the clinical significance of multidrug chemotherapy in colon cancer, the focus of this study was on these chemoresistant mechanisms. In shrunken peritoneal metastasis, we found significant increases in the mRNA levels of Carboxylesterase2 (CE2), which is sensitive to irinotecan-based chemotherapy, although other molecular factors were resistant to both 5-FU and oxaliplatin. On the other hand, we confirmed that the recurrent ovarian tumor showed significant resistance to all three drugs: 5-FU, irinotecan and oxaliplatin. These results directly suggest that cancer heterogeneity both necessitates and limits the use of multidrug chemotherapy in colon cancer.

In conclusion, the complexity of treatment choices and the longer overall survival achievable today clearly warrant an individualized approach towards medical therapy in metastatic colon cancer. Accumulating evidence has demonstrated that colon cancer is heterogenous and complex. However, we believe that detailed genetic and molecular biological analysis of colon cancer will contribute to future effective therapies.

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