

Case Report

# A Rare BRAF Fusion in Advanced Rectal Cancer Treated with Anti-Epidermal Growth Factor Receptor Therapy

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## Keywords

Advanced rectal cancer · Comprehensive genomic profiling · *BRAF* fusion · Anti-epidermal growth factor receptor therapy

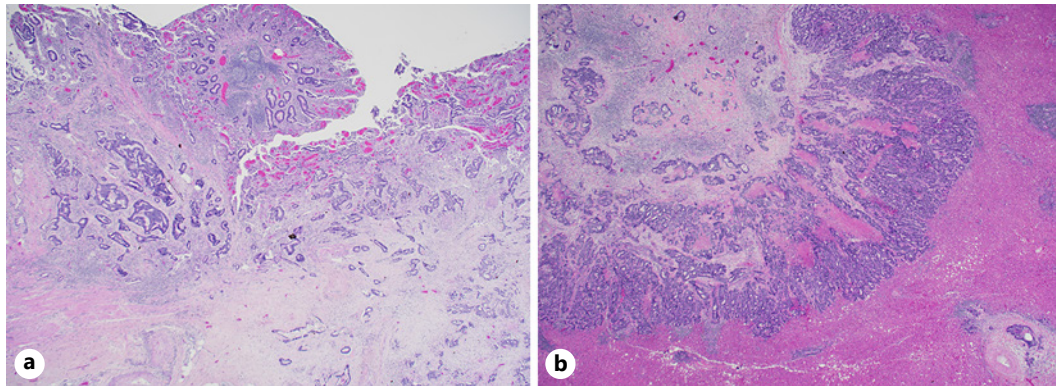
## Abstract

Recently, v-raf murine sarcoma viral oncogene homologue B (*BRAF*) fusions have been identified in multiple cancer types using comprehensive genomic profiling (CGP) assays. *BRAF* fusions are extremely rare, occurring in <0.5% of patients with metastatic colorectal cancer (mCRC). Until now, there is no standard treatment for mCRC with *BRAF* fusions. Here, we report a recurrent colorectal cancer case that harbored an *EXOC4-BRAF* fusion. A 40-year-old female patient with a 2-year history of type 2 diabetes was diagnosed with pathologically confirmed stage IV rectal adenocarcinoma with liver metastasis. She underwent R0 resection after neoadjuvant therapy; however, her disease recurred at multiple metastatic sites (lymph nodes, ovary, and peritoneal gland). A rectal cancer surgical specimen was submitted for CGP (Foundation One) to identify potential targets to develop treatment strategies. An *EXOC4-BRAF* fusion was identified, and she achieved partial response to FOLFOX + panitumumab which is a fully human antibody directed against epidermal growth factor receptor. No *EXOC4-BRAF* fusions in colorectal cancer cases have been reported to date. Further studies investigating molecular mechanisms and novel targeted therapy approaches are required.

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**Fig. 1.** Histopathological examination of the specimen of the resected rectal cancer (**a**,  $\times 30$ ) and the liver metastasis (**b**,  $\times 30$ ) with hematoxylin and eosin stain. Moderately differentiated adenocarcinoma with serosal invasion was diagnosed. Cancer cells are damaged without the destruction of the tumor structure which was consistent with grade 1a effects based on the Japanese Classification.

## Introduction

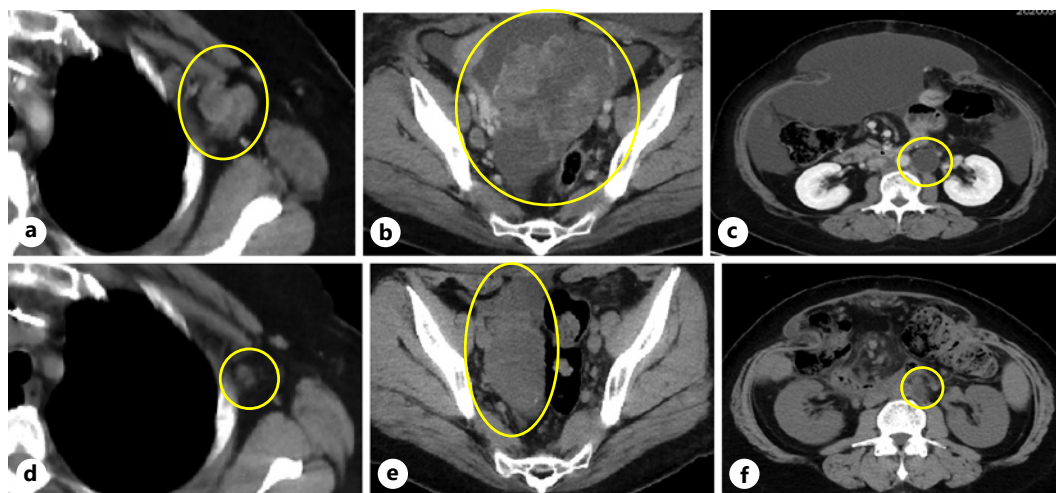
v-raf murine sarcoma viral oncogene homologue B (*BRAF*) encodes a protein kinase that acts through the mitogen-activated protein kinase pathway (RAS/RAF/MEK/ERK pathway) and plays an important role in cell proliferation, differentiation, and survival [1]. *BRAF* alterations have become a major drug target and predictive biomarker in several malignancies [2]. The current understanding of *BRAFV600E* mutations originated in melanoma, where they are well-established biomarkers for cancer treatment and are observed in nearly 50% of cutaneous melanomas [3]. Moreover, in metastatic colorectal cancer (mCRC), *BRAF* mutation which has been identified in 8–10% of cancers is reportedly associated with resistance to anti-epidermal growth factor receptor (EGFR) therapy and a poor prognosis [4].

Recently, fusion genes which have been recognized as important cancer drivers thanks to advances in next-generation sequencing technologies have emerged as oncogenic drivers in several cancer types and represent potential targets for targeted therapy [5]. The *BRAF* gene is also reportedly involved in oncogenic gene fusions, with frequencies of 0.2–3% in some cancers [6]. Until now, *BRAF* fusions in mCRC have been rarely reported; therefore, the impact of fusion partners on the oncogenic role of *BRAF* and chemotherapy efficacy remains unknown.

Herein, we report a rare case of rectal cancer with lymph node and liver metastasis, with an *EXOC4-BRAF* fusion identified by clinical comprehensive genomic profiling (CGP) assay (Foundation One, Foundation Medicine, Cambridge, MA, USA). The patient was treated with anti-EGFR therapy and achieved a considerable clinical benefit.

## Case Report/Case Presentation

A 40-year-old female patient with a 2-year history of type 2 diabetes was diagnosed with pathologically confirmed stage IV (T4aN1M1a) rectal adenocarcinoma (tub2) with liver (S7) metastasis. She received 6 cycles of capecitabine + oxaliplatin (XELOX) + bevacizumab (7.5 mg/kg) therapy as neoadjuvant treatment and underwent R0 resection both for liver metastasis (segmentectomy of S7) and primary rectal cancer in 2018. Pathological examinations revealed a poor response to neoadjuvant chemotherapy, which was consistent with grade 1a effects based on the Japanese Classification of Colorectal Carcinoma (Fig. 1). She then received



**Fig. 2.** Abdominal CT images before anti-EGFR combination therapy. Yellow circles indicate the metastatic sites of axillary lymph node (a), ovary (b), and para-aortic lymph node with ascites (c) Six months after the initiation of anti-EGFR combination therapy. d–f Decrease in size of the metastatic sites and disappearance of ascites (yellow circle). EGFR, epidermal growth factor receptor; CT, computed tomography.

XELOX as adjuvant chemotherapy for 6 months after surgery. However, 2 years after completion of adjuvant chemotherapy, the disease relapsed with multiple metastases in lymph nodes and ovary. Subsequently, she received FOLFIRI (irinotecan in combination with 5-fluorouracil (5-FU)/leucovorin) + bevacizumab therapy as palliative chemotherapy. A follow-up computed tomography scan after 3 FOLFIRI + bevacizumab cycles indicated that the size of lymph node metastases and ovarian metastasis had increased and moderate ascites extending from the pelvic to lower abdominal cavity due to the peritoneal metastases occurred. Because of a poor response to FOLFIRI + bevacizumab therapy, laboratory tests showed that carcinoembryonic antigen serum levels had increased from 40.0 to 52.0 ng/mL (normal range: 0–4.0 ng/mL) and carbohydrate antigen 19-9 serum levels had increased from 129 to 192 U/mL (normal range: 0–40 U/mL) when compared to before treatment commencement.

We also analyzed the mutational status of *RAS* and *BRAF* genes using polymerase chain reaction from surgical specimens of rectal cancer. No *RAS* and *BRAF* mutations were identified. A polymerase chain reaction-based assay for microsatellite instability did not reveal a high microsatellite instability profile. To explore potential targets for clinical trials or novel treatment strategies, rectal cancer surgical specimens were submitted for CGP to identify alterations in specific genes (Foundation One).

The CGP assay revealed mutations in *ABL1* (R712H), *APC* (R283), and *TP53* (A138V), and a *BRAF* fusion (*EXOC4-BRAF*) was also identified. The tumor mutation burden was 1.22 mut/Mb. At this point, no clinical trials were available for the patient to participate in; therefore, she continued with systematic chemotherapy. Given the presence of a primary left-sided colorectal tumor and no *RAS* and *BRAF* mutations, she received second-line palliative chemotherapy with FOLFOX-6 (oxaliplatin in combination with 5-FU/leucovorin) plus panitumumab which is a fully human antibody directed against EGFR. After six cycles, the patient achieved partial responses based on Response Evaluation Criteria in Solid Tumors version 1.1. Her ovarian metastasis, lymph nodes metastases, and massive ascites were decreased, and tumor markers were also decreased (carcinoembryonic antigen: 17.9 ng/mL and carbohydrate antigen 19-9: 22 U/mL) (Fig. 2, 3). Over 12 months, the patient continued on FOLFOX-6 plus panitumumab therapy, with the best response of partial response.

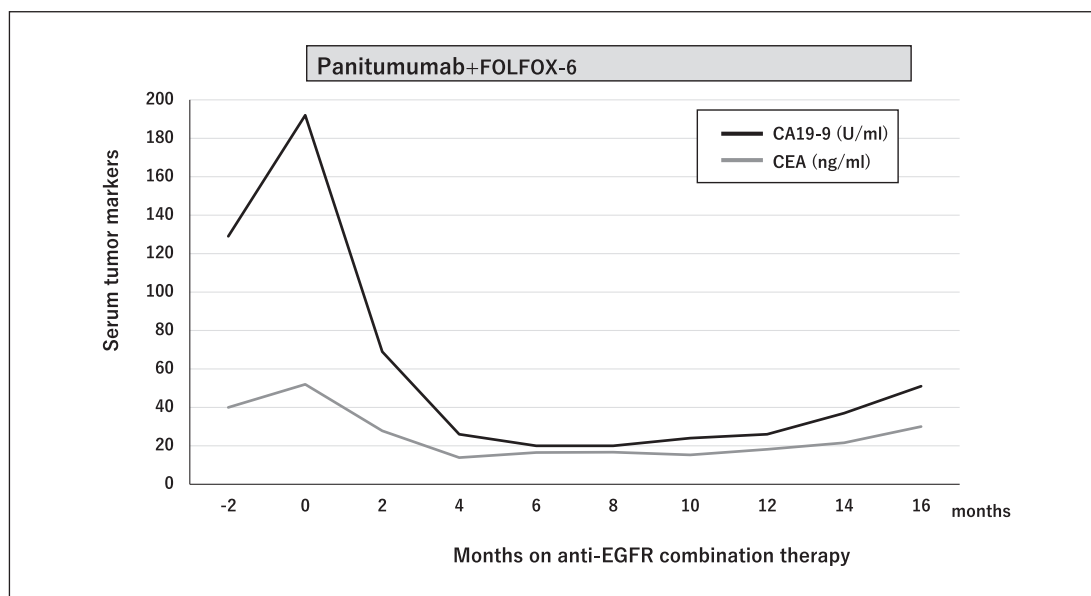


Fig. 3. Changes in serum level of tumor markers.

Table 1. Summary of cases with BRAF fusion in colorectal cancer

Author	Types of BRAF fusion in colorectal cancer
Ross et al. [6]	<i>MKRN1-BRAF</i> <i>TRIM24-BRAF</i> <i>AGAP3-BRAF</i>
Kloosterman et al. [10]	<i>AGAP3-BRAF</i> <i>TRIM24-BRAF</i> <i>DLG1-BRAF</i>
Current case	<i>EXOC4-BRAF</i>

## Discussion

Since the first *BRAF* fusion case was identified in papillary thyroid carcinoma in 2005 [7], hundreds of tumors with the *BRAF* kinase domain fused to one or more partner genes (110 to date) have been identified in several cancer tumor types such as melanoma, lung, and prostate cancer [6, 8, 9]. In mCRC, *BRAF* fusions are rarely identified, that is, <0.5%, according to a previous report [6]. Until now, some fusion partners of *BRAF* in mCRC were reported [6, 10] (Table 1); however, the prognostic and biological implications of *BRAF* fusions are unclear.

In terms of anti-EGFR therapy responses, the presence of *BRAFV600E* mutations was previously identified as negative response predictors in mCRC, based on sub-analysis data from phase III clinical trials [11]; however, it is currently unclear whether *BRAF* fusions in mCRC affect chemotherapeutic efficacy or prognosis.

To our knowledge, this is the first rare case report of responses to anti-EGFR therapy in mCRC where an *EXOC4-BRAF* fusion gene was identified. The exocyst complex component 4 (*EXOC4*) gene is located on the same chromosome as *BRAF* and is associated with insulin-stimulated glucose transport related to type 2 diabetes [12].

According to a previous preclinical study [13], specific *BRAF* fusion genes (*AGAP3-BRAF*, *DLG1-BRAF*, and *TRIM24-BRAF*) were suggested to be relevant to the resistance of EGFR targeted therapy, same as *BRAFV600E* mutation; on the other hand, other novel *BRAF* fusions were reported not to encode protein partners with obvious dimerization motifs associated with resistance of RAF inhibition [9]. It is unclear if these other novel fusions influenced the sensitivity to anti-EGFR therapy. In our case, the clinical features of this *BRAF* fusion may be similar to non-*V600E* *BRAF* mutations in mCRC, especially for class 3 *BRAF* mutations which were reportedly sensitive to anti-EGFR therapy owing to *RAS* dependency [14].

For melanoma expressing *BRAF* fusions, remarkable clinical response was achieved by MEK inhibition but not by BRAF inhibition [15]. For MEK inhibition, some *BRAF* fusions such as *PAPSS1-BRAF*, *TRIM24-BRAF*, and *FAM114A2-BRAF*, *ATG7-BRAF* in melanoma, as well as *ZC3HAV1-BRAF* and *FAM114A2-BRAF* in thyroid cancer among various types of fusions which likely produce constitutively activated BRAF dimers independently of *RAS* were reported to be sensitive to MEK inhibitors [15]. To date, there is no data on the effective therapy for mCRC with *BRAF* fusion, due to the rarity of this disease.

## Conclusion

In conclusion, a comprehensive targeted therapy has not been established for mCRC patients harboring *BRAF* fusions due to disease rarity. Further studies are required to investigate the clinical features of different *BRAF* fusions, and similarly, additional research is needed to develop targeted therapeutic strategies for this fusion in mCRC.

At the time of this report, the patient was on the next palliative chemotherapy as salvage line therapy with stable disease 40 months after the first diagnosis. Compassionate use program based on JAPAN's system for MEK inhibitor is now being discussed.

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## Statement of Ethics

The case was approved by the Clinical Ethics Committee of the Osaka National Hospital. The written informed consent form was obtained from this patient to publish her case (including publication of images).

## Conflict of Interest Statement

All the authors have no conflicts of interest to declare.

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The authors declare that there was no funding for this case report.

### Author Contributions

H.H. involved in the case, collected the data of the case, and wrote the manuscript. M.M. and K.M. were responsible for patient diagnosis and treatment. E.M. and H.I. helped in writing and editing the case report. All the authors critically revised the manuscript, approved the final manuscript, and agreed to be accountable for all aspects of the work.

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