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Anti-epidermal growth factor receptor treatment for patients with Neo*RAS* wild-type metastatic colorectal cancer: a case report of two cases

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***Abstract*:** The Neo*RAS* phenomenon is defined as the conversion of tumor *RAS* status from mutant-type (MT) to wild-type (WT) after systemic chemotherapy in metastatic colorectal cancer (mCRC). Cetuximab, an anti-epidermal growth factor receptor (EGFR) antibody, is effective in patients with *RAS* WT mCRC but ineffective in those with *RAS* MT mCRC; however, its outcome in patients with Neo*RAS* WT mCRC is unclear. Herein, we report two cases of Neo*RAS* WT mCRC that responded clinically to anti-EGFR treatment. The first was a 40-year-old man with synchronous peritoneal metastatic rectosigmoid cancer. The first *RAS* testing on tumor tissue revealed a *KRAS* G12C mutation, which was converted to *RAS* WT after two lines of chemotherapy, as assessed by liquid biopsy. After initiating irinotecan plus cetuximab treatment, a computed tomography (CT) scan revealed that malignant ascites had resolved. The treatment was discontinued after 4 months because of disease progression. The second was a 68-year-old male patient with synchronous liver metastasis from sigmoid colon cancer. The *KRAS* G12D mutation, initially detected in tumor tissue, was not detected by liquid biopsy after six lines of chemotherapy. Cetuximab monotherapy was initiated, and the liver metastases shrank significantly. The patient continued cetuximab monotherapy for 8 months without disease progression. Our cases demonstrate the efficacy of anti-EGFR therapy for Neo*RAS* WT mCRC and highlight the importance of capturing the gene mutation profile throughout the clinical course for optimal treatment selection.

Keywords: case report, chemotherapy, colorectal cancer, EGFR inhibitors, Neo*RAS*, precision oncology

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Introduction

Metastatic colorectal cancer (mCRC) is the second leading cause of cancer death worldwide.[1] Its prognosis remains poor, but the development of molecular targeting agents has extended the survival time of patients with mCRC. Currently, it is essential to evaluate the multiple gene alterations of an individual's tumor and select appropriate molecular targeting agents for the treatment of mCRC.

RAS mutations (*KRAS* or *NRAS* exons 2, 3, and 4) are found in approximately 50% of patients with mCRC.[2] Previous studies have shown that anti-epidermal growth factor receptor (EGFR) antibodies, cetuximab and panitumumab, have lower survival benefits for patients with *RAS* mutant-type (MT) mCRC than for those with *RAS* wild-type (WT) tumors.[3,4] Because *RAS* mutations are negative predictors of anti-EGFR antibody efficacy, international practice guidelines recommend *RAS* testing prior to initiating anti-EGFR therapy in patients with mCRC.[5–7] Furthermore, the *KRAS* G12C mutation, which occurs in 3% of patients with mCRC,[8] has been

identified as not only a negative predictor of anti-EGFR antibodies but also a novel treatment target for solid tumors, including mCRC.[9,10]

Previously, *RAS* testing was typically performed on tumor tissue obtained through biopsy or surgery. However, such tumor sampling is usually invasive and difficult to repeat. Thus, monitoring *RAS* mutational status has been difficult during chemotherapy. In addition, the results of *RAS* testing using tumor biopsy may be limited by spatial tumor heterogeneity. Tumor heterogeneity refers to the notion that a single tumor consists of numerous subclone cells and that analysis using a portion of the tumor tissue may not reflect the genetic abnormalities of the entire tumor within the patient.[11]

Recent studies have demonstrated that analyzing circulating tumor DNA (ctDNA) in blood samples is a remarkable surrogate for tumor biopsy for detecting mutations and overcoming spatial tumor heterogeneity. Because such liquid biopsy using ctDNA is also a noninvasive method compared to conventional tissue sampling, it allows for monitoring of gene mutational status changes through the clinical course.[12,13] In previous clinical trials, a comparison between the use of beads, emulsion, amplification, and magnetic digital polymerase chain reaction (PCR; BEAMing) technology to determine *RAS* mutational status in plasma ctDNA and the reference method of tumor tissue DNA revealed concordance rates ranging from 86% to 93%.[13–17]

The development of liquid biopsy using ctDNA has gradually revealed dynamic changes in *RAS* mutational status during chemotherapy in patients with mCRC. Several studies have revealed that the *RAS* mutational status of some patients with mCRC changed from *RAS* WT to MT during anti-EGFR therapy.[18] The emergence of *RAS* MT subclones is well described as a result of clonal evolution under treatment-induced selection pressure, resulting in acquired resistance to anti-EGFR treatment.[19–21]

On the other hand, reversion of *RAS* MT to WT was thought to be rare because of the expected evolutionary advantage of *RAS* MT clones during tumor evolution.[22] However, the reversion of *RAS* MT to *RAS* WT during chemotherapy, known as the Neo*RAS* phenomenon, has gained popularity in recent years. The mechanisms of the Neo*RAS* phenomenon remain unclear, and its frequency varies greatly among reports, ranging from 10.7% to 83.3%.[23–29] It is worth considering whether patients with Neo*RAS* WT mCRC can benefit from anti-EGFR treatments as much as those with *RAS* WT mCRC, because treatment options for patients with *RAS* MT mCRC are limited in comparison to those for patients with *RAS* WT mCRC. However, few reports have described the clinical outcomes of anti-EGFR treatments for patients with Neo*RAS* WT mCRC. Herein, we report two cases of Neo*RAS* WT mCRC that achieved clinical response to anti-EGFR therapy, as well as a literature review.

Case 1: A 40-year-old male patient with synchronous peritoneal metastasized rectosigmoid cancer

A 40-year-old male patient presented with constipation and was diagnosed with synchronous peritoneal metastasized rectosigmoid cancer (T4aN2bM1c Stage IVC according to the Union for International Cancer Control TNM, 8th edition). Due to the rectal obstruction, an emergency laparoscope-assisted high anterior resection was performed. The primary tumor was pathologically evaluated and showed a well-differentiated adenocarcinoma. Mutational analysis of the primary tumor tissue was performed using the MEBGEN RASKET-B kit™ and the PCR-rSSO method (Medical & Biological Laboratories Co., Tokyo, Japan) and revealed the *KRAS* G12C mutation, *NRAS*, and *BRAF* WT. Microsatellite instability (MSI) testing using a PCR-based method was negative. In addition to the aforementioned gene alterations, genetic analysis of primary tumor tissue using the OncoGuide™ NCC Oncopanel System (Sysmex Corporation, Hyogo, Japan) revealed several oncogenic gene alterations (Figure 1). Following surgery, the patient was started on capecitabine, oxaliplatin, and bevacizumab (CapOX+BEV) as first-line systemic chemotherapy. Partial response (PR) was the best tumor response as assessed by Response Evaluation Criteria in Solid Tumors 1.1. Fifteen months after the initiation of CapOX+BEV, a computed tomography (CT) scan revealed an increase in ascites and suggested disease progression. Subsequently, the fluorouracil, leucovorin, irinotecan, and ramucirumab (FOLFIRI+RAM) regimen was initiated as second-line chemotherapy, but it was discontinued 1.5 months later due to disease progression. At the end of each first- and second-line chemotherapy, a liquid biopsy was performed using the

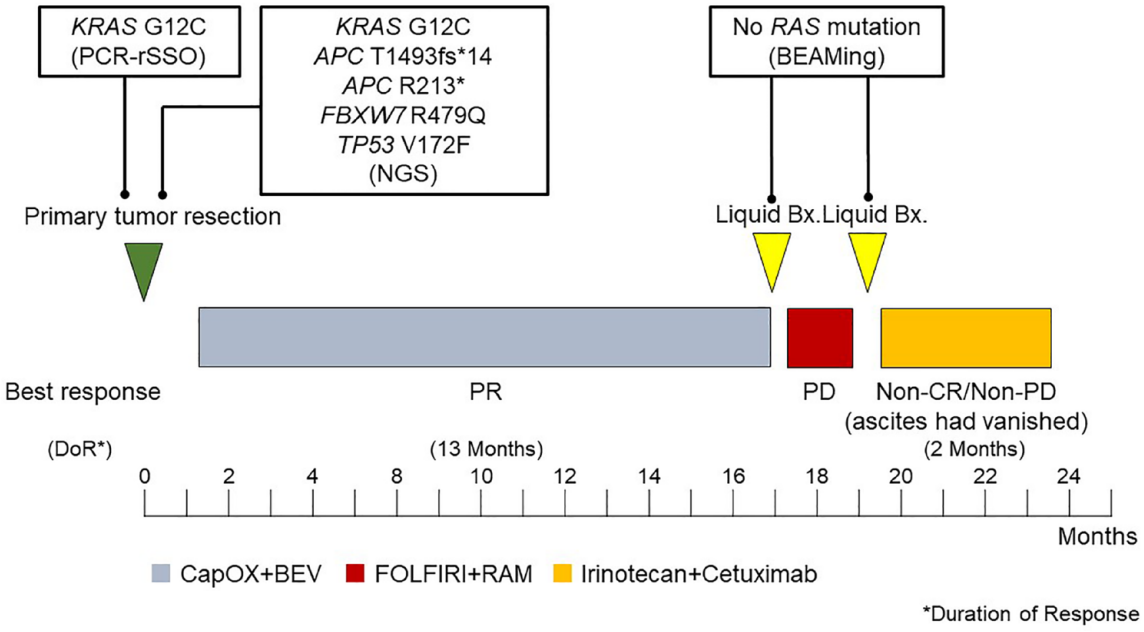

Figure 1. The clinical course of the case 1.

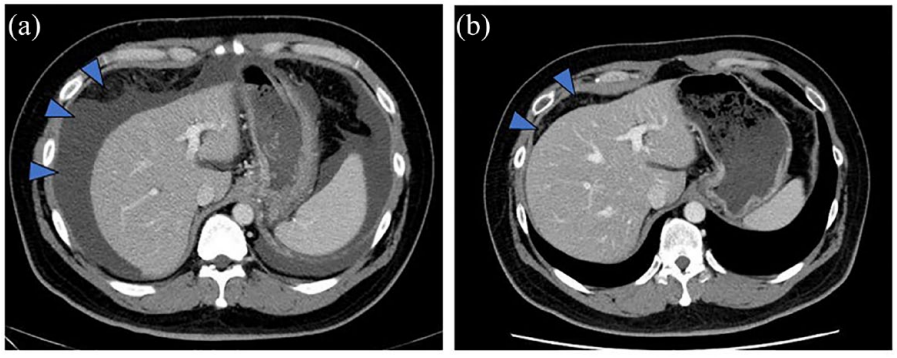

Figure 2. (a) The CT scan before administration of irinotecan plus cetuximab and (b) After 2 months, a CT scan revealed the ascites had vanished (arrowhead).

OncoBEAM™ RAS CRC Kit (Sysmex Corporation, Hyogo, Japan), which is based on BEAMing technology. Although the *KRAS* G12C mutation was detected *via* mutational testing on primary tumor tissue, neither of the liquid biopsies detected the *RAS* mutation. Irinotecan plus cetuximab was started as third-line chemotherapy with the patient's consent. After 2 months, a CT scan revealed that the ascites had vanished [Figure 2(a) and (b)]. There were no severe adverse events during the treatment. Four months after initiating cetuximab, peritoneal metastasis progressed. Subsequently, trifluridine/tipiracil (FTD/TPI) +BEV and regorafenib were started, both of which caused disease progression. This patient is currently receiving the best supportive care.

Case 2: A 68-year-old male patient with synchronous hepatic metastasized sigmoid colon cancer

A 68-year-old male patient was diagnosed with sigmoid colon cancer with multiple liver and lymph node metastases (T3N2bM1b Stage IVB according to the Union for International Cancer Control

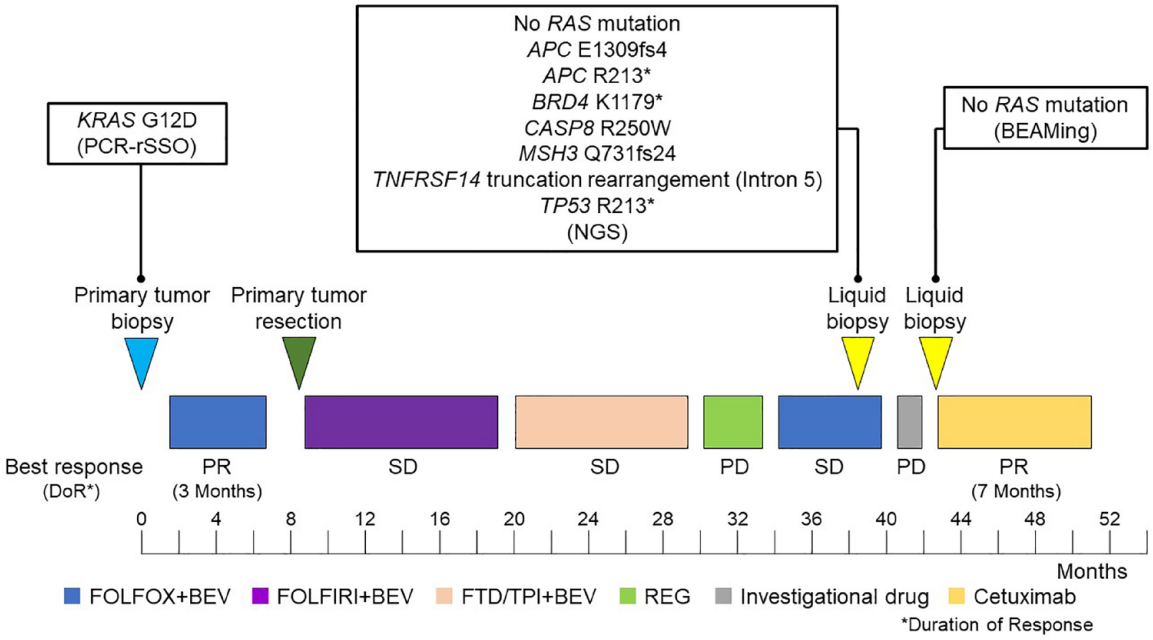

Figure 3. The clinical course of the case 2.

TNM, 8th edition). The primary tumor was pathologically diagnosed as a well-differentiated adenocarcinoma. Mutational analysis of the primary tumor tissue using the MEBGEN RASKET-B kit revealed the *KRAS* G12D mutation, *NRAS*, and *BRAF* WT. MSI testing using a PCR-based method yielded a negative result (nonMSI-High). After the primary tumor was resected, the patient was treated with six lines of systemic chemotherapy: FOLFOX (fluorouracil, leucovorin, and oxaliplatin) plus BEV, FOLFIRI+BEV, FTD/TPI+BEV, regorafenib, FOLFOX+BEV reintroduction, and investigational immunotherapy. The tumor response was PR during FOLFOX+BEV application as first-line chemotherapy. However, other treatment regimens did not result in tumor shrinkage, and all ended in disease progression. During the FOLFOX+BEV reintroduction treatment, comprehensive genomic analysis was performed using the Foundation One Liquid CDx™ Assay (Foundation Medicine, Cambridge, MA, USA). It revealed several actionable genomic alterations and a high tumor mutation burden, but not the *KRAS* G12D mutation (Figure 3). After the completion of investigational immunotherapy, the *KRAS* mutational status was reassessed using the OncoBEAM™ RAS CRC Kit, which revealed *RAS* WT, and cetuximab monotherapy was initiated as seventh-line therapy with the patient's consent. After 3 months, a CT scan revealed remarkable shrinkage of the liver metastasis [Figure 4(a) and (b)]. The patient continued cetuximab monotherapy for 8 months without disease progression. No severe adverse events were observed during the treatment. However, he died of cancer progression 10 months after cetuximab initiation.

Discussion

The use of anti-EGFR antibodies for Neo*RAS* WT mCRC remains controversial, but it is worth investigating because treatment options for *RAS* MT mCRC are limited. Several previous studies suggested the effectiveness of anti-EGFR antibodies in Neo*RAS* WT mCRC.[23,30–34] However, all of these findings were obtained in combination with cytotoxic chemotherapy, and the extent to which anti-EGFR antibodies contributed to these findings is unclear. In Case 2, remarkable tumor shrinkage was observed following the initiation of cetuximab monotherapy. Our report is the first to show efficacy of anti-EGFR antibody monotherapy in Neo*RAS* WT mCRC. It more clearly suggests that anti-EGFR antibodies are effective, at least in some patients with Neo*RAS* WT mCRC. Several prospective clinical trials to evaluate the treatment outcomes of anti-EGFR antibodies for Neo*RAS* WT mCRC are currently underway, with results expected.[35]

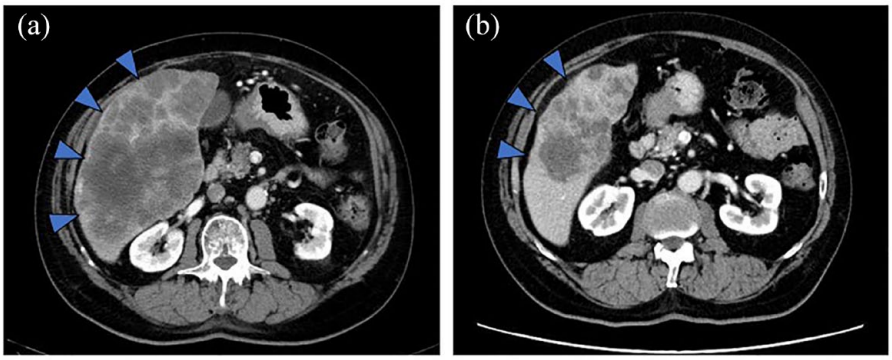

Figure 4. (a) The CT scan before administration of cetuximab monotherapy and (b) After 3 months, a CT scan revealed remarkable shrinkage of the liver metastasis (arrowhead).

In our cases, there were discrepancies in the results of *RAS* gene mutation tests performed at different times. However, all the tests were properly performed according to the manufacturer's instructions, and we do not believe that the discrepancies were caused by the testing technique. Though the mechanisms of such Neo*RAS* phenomenon are unclear, they may be explained by intratumor heterogeneity and chemotherapy-induced clonal evolution. In other words, systemic chemotherapy eliminates the initially predominant *RAS* MT subclones while increasing the proportion of the minor clone, the *RAS* WT subclone, resulting in the Neo*RAS* phenomenon. Indeed, Klein-Scory *et al.* demonstrated that *RAS* WT clones disappeared rapidly during chemotherapy and converted to *RAS* WT clones in more than 90% of patients with *RAS* MT mCRC who responded to chemotherapy.[36] Long *RAS* testing intervals,[37] as well as a good response to chemotherapy prior to second sampling,[32,36] are significantly associated with the Neo*RAS* phenomenon, suggesting that clonal evolution by chemotherapy causes the Neo*RAS* phenomenon. From this perspective, the *RAS* MT subclone might be present in plasma samples from patients with Neo*RAS* WT tumors below the ctDNA assay limit of detection.[30] However, even if this were the case, it would not negate the efficacy of anti-EGFR treatment in Neo*RAS* WT mCRC because it has been demonstrated that patients with a low *RAS* mutant fraction (0.1–5%) might benefit from the addition of cetuximab to chemotherapy.[4,38] Both of our two cases achieved PR according to the Response Evaluation Criteria in Solid Tumors during first-line chemotherapy, and the sampling interval for *RAS* testing was >15 months. The clinical courses of our cases support the existence of a link between chemotherapy-induced clonal evolution and the Neo*RAS* phenomenon. For future investigation, gene mutation analysis with deep sequencing using ctDNA at the baseline or tumor tissues from multiple lesions may be useful to clarify the involvement of intra-tumor heterogeneity and clonal evolution in the Neo*RAS* phenomenon.

Our two cases highlight the importance of repeating genetic analysis throughout the treatment course and understanding gene mutational profiles in real-time for patients with mCRC. In Case 1, the *KRAS* G12C mutation was detected in tumor tissue samples prior to chemotherapy induction, and the effectiveness of *KRAS* G12C inhibitors was anticipated.[9,10] However, a liquid biopsy after first-line chemotherapies revealed that the tumor's *RAS* status had converted to Neo*RAS* WT. Though the effectiveness of *KRAS* G12C inhibitors for Neo*RAS* WT mCRC is unclear, *RAS* testing just prior to treatment may be desirable for the indication of *KRAS* G12C inhibitors, taking the Neo*RAS* phenomenon into account. In Case 2, comprehensive genetic analysis prior to cetuximab administration revealed no negative predictors of anti-EGFR antibody efficacy, such as *RAS* MT, *HER2* amplification, or *BRAF* V600E MT.[39] This case demonstrates that comprehensive genetic mutation analysis may be able to efficiently predict which patients will benefit from highly effective anti-EGFR antibodies.

In conclusion, we presented two cases of mCRC with RAS gene status divergence between tissue and blood sample testing. Both were initially diagnosed with *RAS* MT mCRC using primary

tumor tissues, but liquid biopsy after systemic chemotherapy revealed *RAS* WT status. These patients with Neo*RAS* WT mCRC responded clinically to anti-EGFR therapy. Though the etiology and clinical significance of the Neo*RAS* phenomenon are not fully understood, it has been demonstrated that anti-EGFR antibodies are effective in some patients with Neo*RAS* WT mCRC. Furthermore, our cases highlight the importance of capturing the gene mutation profile throughout the clinical course, rather than just once, for optimal treatment selection.

Declarations

Ethics approval and consent to participate

Not applicable. Since the case report is not subject to ethical review under the 'Ethical Guidelines for Life Science and Medical Research Involving Human Subjects', it was waived from review by the Ethical Review Committee for Life Science and Medical Research at Hokkaido University Hospital.

Consent for publication

All patients provided consent for publication of this manuscript.

Author contributions

Kazuaki Harada: Data curation; Writing – original draft.

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Takeaki Nakamura: Writing – review & editing.

Shiho Kaneko: Writing – review & editing.

Koichi Ishida: Writing – review & editing.

Naoya Sakamoto: Writing – review & editing.

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Competing interests

The authors declare that there is no conflict of interest.

Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author.

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