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BRAF Mutation Heterogeneity Detected Using Circulating Tumor DNA Sequencing in Synchronous Colon Cancer: A Case Report

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Abstract. *Background/Aim: Synchronous colorectal cancer, which occurs in approximately 4.8-8.4% of all colorectal cancers, has a genetic profile with a higher rate of v-raf murine sarcoma viral oncogene homolog B1 (BRAF) mutation and microsatellite instability-high than solitary colorectal cancer. However, little information is available on heterogeneity among tumor lesions because of difficulty in performing genetic tests in all lesions in clinical practice. Case Report: A 44-year-old man presented with multiple recurrent lung metastases 42 months after the endoscopic resection of early stage synchronous ascending and sigmoid colon cancers. The genetic testing of sigmoid colon cancer tissue samples, their state being more advanced than that of ascending colon cancer, revealed a v-Ki-ras 2 Kirsten rat sarcoma viral oncogene homolog mutation (G13C) and BRAF wild type. However, the tumor was refractory to initial chemotherapy and rapidly progressed to new liver metastases. Therefore, we suspected that there may be biological heterogeneity between the primary sigmoid colon lesion and liver metastases. Next, we performed next-generation sequencing on circulating tumor DNA from the patient's plasma (Foundation One Liquid CDx®), which revealed the V600E mutation of BRAF, suggesting that there was genetic heterogeneity among the synchronized primary lesions, one of which was responsible for the chemo-refractory rapid-growing liver metastases. Conclusion: Genetic profiling with liquid biopsy at the time of recurrence and metastasis may be useful in patients with multiple synchronous cancers because there is less heterogeneity between primary and metastatic sites.*

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Synchronous colorectal cancer has been reported to occur in approximately 4.8-8.4% of all colorectal cancers (1, 2). Furthermore, compared with solitary colorectal cancer, it is more likely to harbor genetic profiles such as v-raf murine sarcoma viral oncogene homolog B1 (*BRAF*) mutations and microsatellite instability (MSI)-high (3). However, little information is available regarding the genetic heterogeneity of these lesions, and it is difficult to examine the genetic profile of each lesion in clinical practice. Gene profile testing using liquid biopsies has the advantage of being less susceptible to spatial and temporal heterogeneity (4). Here, we report a patient with recurrent synchronous colon cancer whose liquid biopsy detected a *BRAF* mutation, which was not detected by the genome testing of one of the primary tumors.

Case Report

A 44-year-old man with no prior medical history presented to our hospital with a positive fecal occult blood test. His family history included breast cancer in the mother, colon cancer in the paternal grandfather, and rectal cancer in the maternal

grandmother. He was diagnosed with multiple colon adenocarcinomas of TisN0M0 (Stage 0) in the ascending colon and T1bN0M0 (Stage I) in the sigmoid colon, according to the UICC TNM classification 8th edition, by colonoscopic evaluation. Endoscopic submucosal dissection and endoscopic mucosal resection (EMR) were performed on the ascending and sigmoid colon adenocarcinomas, respectively. The pathological examination revealed that the ascending colon lesion was tubular adenocarcinoma with no vascular or lymphatic invasion (Tis), and the sigmoid colon lesion was tubular adenocarcinoma with a submucosal invasion of 6,000 μm and mild positive vascular invasion (T1). The pathological findings of the sigmoid colon lesion were determined to be high–risk factors for metastasis, and additional surgical resection was performed one month after the EMR, which revealed complete resection (R0). Forty-two months after the surgical resection, computed tomography (CT) revealed multiple nodules in both lungs, suggesting the recurrence of lung metastases (Figure 1).

Genetic testing for rat sarcoma (*RAS*), *BRAF*, and MSI using polymerase chain reactions was performed on a tissue sample from the sigmoid colon, which had a more advanced state than the ascending colon. The tests revealed a Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutation (G13C), *BRAF* wild type and microsatellite stability.

The patient received first-line palliative chemotherapy with SOX (tegafur/gimeracil/oteracil 120 mg orally from days 1 to 14, oxaliplatin 130 mg/m^2 intravenously on day 1) and bevacizumab (BV) (7.5 mg/kg intravenously on day 1) on a tri-weekly schedule. After two cycles, the patient experienced diarrhea and abdominal pain, probably due to the tegafur/gimeracil/oteracil, classified as Grade 2 according to the Common Terminology Criteria for Adverse Events version 5.0. Due to these adverse events, the regimen was changed from SOX + BV to mFOLFOX6 (oxaliplatin 85 mg/m^2 intravenously on day 1, fluorouracil 400 mg/m^2 bolus infusion intravenously on day 1, followed by 2,400 mg/m^2 continuous infusion intravenously for 46 hours on days 1 to 3, and leucovorin 200 mg/m^2 intravenously on day 1) + BV (5 mg/kg on day 1) every 2 weeks. After two cycles of mFOLFOX6 + BV, CT showed that multiple lung metastases had shrunk, but that new lesions had appeared in the liver (Figure 2). The chemotherapy regimen was changed to FOLFIRI (irinotecan 150 mg/m^2 intravenously on day 1, fluorouracil 400 mg/m^2 bolus infusion intravenously on day 1, followed by 2,400 mg/m^2 continuous infusion intravenously for 46 hours on days 1 to 3, and leucovorin 200 mg/m^2 intravenously on day 1) + ramucirumab (8 mg/kg intravenously on day 1) every 2 weeks as subsequent second-line chemotherapy. At the same time, we performed next-generation sequencing (NGS) on circulating tumor DNA (ctDNA) from the patient's plasma (Foundation One Liquid CDx®; Foundation Medicine, Inc., Cambridge, MA, USA). In the genetic test, a *BRAF* mutation [c.1799T>A, pV600E, allele frequency 1.2% (83/6,951)] was identified. RAS wild type was also identified and the MSI status showed microsatellite stability. After four cycles of FOLFIRI + ramucirumab, follow-up CT imaging showed stable disease. Based on the results of the ctDNA NGS analysis, encorafenib (300 mg orally daily) + cetuximab (400 mg/m^2 as an initial dose, followed by 250 mg/m^2 weekly intravenous administration) was started. Two months after treatment initiation, the patient died due to disease progression.

Discussion

The current case presented multiple synchronous colon cancers of the sigmoid and ascending colons, and recurrence with multiple lung metastases 42 months after surgical resection. Initially, we believed that the more advanced primary site of the sigmoid colon had recurred; therefore, a genetic test was performed using sigmoid colon tissue. This testing revealed a *KRAS* mutation but *BRAF* wild type. We initiated first-line chemotherapy, but the tumor was refractory to this treatment, and liver metastases appeared rapidly as a new lesion. We suspected that there may have been biological heterogeneity between the primary lesion and liver metastases because the patient's clinical course deviated from the general course predicted by the genetic profile of the primary lesion. Liquid biopsy also revealed a *BRAF* mutation.

Consequently, when there are discrepancies between tissue and liquid biopsy results, several possibilities exist as to why. One is that the *BRAF*-mutated cells responsible for recurrence may have originated from another primary tumor of the ascending colon. Shu *et al*. reported an 87% concordance rate between ctDNA and tumor tissue in various cancer types, including colorectal cancer (5). Therefore, it is unlikely that the present *BRAF* mutation was undetectable in the genetic testing of the tumor sample. Furthermore, *BRAF*-mutated colon cancer is generally located on the right side (6) and the clinical course of metastatic recurrent *BRAF*-mutated colorectal cancer is often characterized by rapid progression and resistance to conventional chemotherapy (7), which is consistent with the current case. Another consideration is that the present *BRAF* mutation may have been added to the recurrent metastases, despite the primary tumor having *BRAF* wild type. A meta-analysis found an 8% discordance rate in *BRAF* mutations between primary and metastatic sites (8); however, most of the papers included in the analysis reported 100% concordance, and only a few studies found discordance, suggesting that publication bias may be inherent. In general, *BRAF* mutations occur early in the process of carcinogenesis (9); therefore, it is unlikely that *BRAF* mutations arise as secondary events in metastases.

The approach presented in this case report had several limitations. First, a genetic test was performed using the primary site of the ascending colon, providing definitive proof that *BRAF*-mutated cells originated from this location. Second, the allele frequency of the *BRAF* mutation in the liquid biopsy

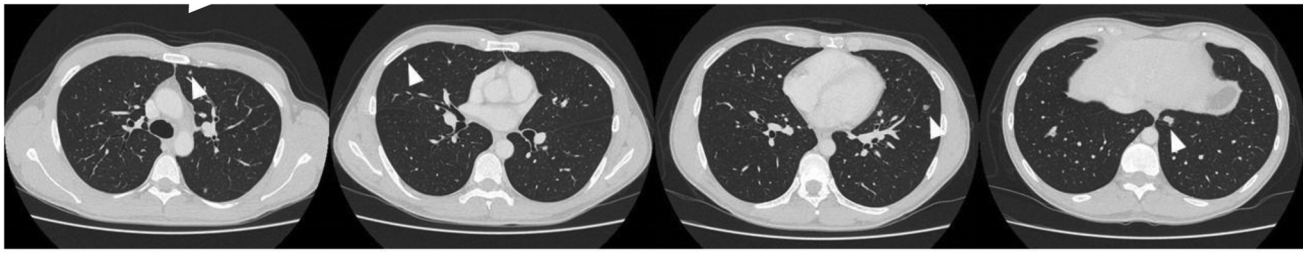

Figure 1. *Multiple bilateral recurrent lung metastasis revealed using computed tomography (white arrowhead).*

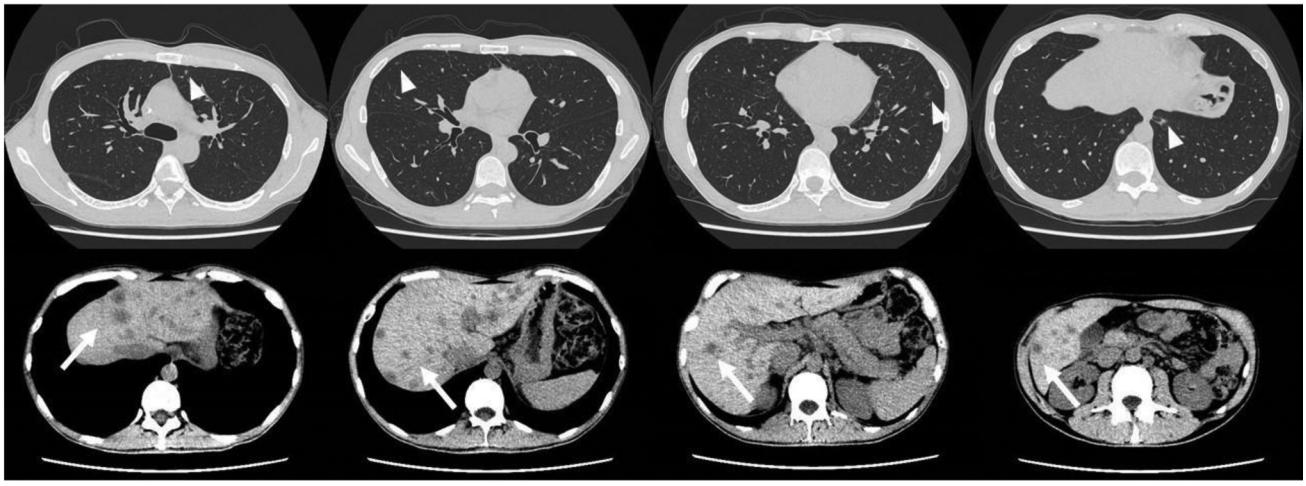

Figure 2. *Multiple lung metastasis shrunk (white arrowhead), but new liver metastatic lesions (white arrow) appeared after the administration of oxaliplatin + fluorouracil bolus + fluorouracil continuous infusion + leucovorin (mFOLFOX6) + bevacizumab.*

was low at 1.2%, and the genetic profile of the liver metastases was not evaluated. Lastly, despite the presence of clinical findings that appeared to be consistent with *BRAF*-mutated colon cancer, treatment with the BRAF inhibitor encorafenib + cetuximab was not effective in mitigating the liver metastases. These findings suggest that *BRAF*-mutated clones may not be the primary drivers of liver metastasis.

In conclusion, genetic profiling tests using liquid biopsies may be useful in patients with multiple synchronous cancers at the time of recurrence and metastasis because there is less tumor heterogeneity between primary and metastatic sites. Additionally, each primary tumor may have a different genetic profile, and it is difficult to perform genetic testing for all primary tumors in clinical practice.

Conflicts of Interest

All Authors have no competing financial interests to declare, except for Y. Miura, who reports receiving honoraria from ONO Pharmaceutical, Bristol-Myers Squibb, MSD, Eisai, and Takeda separate from the submitted work.

Authors' Contributions

Haruka Kono and Taro Yamanaka identified the concept and wrote the original draft. Yuji Miura edited and supervised the writing of the draft. All Authors have read and approved the final version of the article.

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