Comprehensive next-generation sequencing (NGS) reveals multiple primary colorectal carcinoma (MPCC)

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Case Report

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Abstract

Multiple primary colorectal carcinoma (MPCC) is a rare clinical disease, which is difficult to distinguish from metastatic disease by histopathological approaches. Next-generation sequencing (NGS) have been applied to solve the problem of identifying multiple primary cancers. In the study, a rare case of a 63-year-old male patient was diagnosed with MPCC by targeted NGS, which was firstly missed diagnosed by radiological evaluation. The separated two tumors on the surface of the colorectal shared no same genomic alterations. Based on the wild-type KRAS detected in the unrectected tumor, the patient benefited from the EGFR inhibitor (cetuximab), but developed novel mutations including KIF5B-RET fusion, which provides possible resistance mechanism to anti-EGFR therapy. Our case highlights the necessity of using genetic testing for primary tumor diagnosis and the application of serial plasma ctDNA profiling for dynamic disease monitoring.

Introduction

Colorectal cancer (CRC) is one of the most lethal and prevalent malignancies in the world, and around half of CRC patients eventually develop metastatic CRC (mCRC) (1). However, the incidence of multiple primary colorectal carcinoma (MPCC) is still rare (between 1.1% and 8.1%) (2). MPCC is defined as two or more primary colorectal carcinomas detected in a single individual occurring either synchronously or metachronously (3). Preoperative detection of multiple primary cancers is very important when planning treatments. Nevertheless, the current diagnostic criteria were still unable to identify some MPCC patients to make appropriate treatment and follow-up plans (4). Therefore, molecular testing and genomic profiling have been proposed in recent studies to reduce the rate of misdiagnosis or missed diagnosis for multiple primary cancers (5). Herein, we reported a case with MPCC, which was escaped diagnosis via imaging but diagnosed via next-generation sequencing (NGS), showed completely distinct mutation patterns between the two tumors on the surface of the colorectal. Besides, we identified a KIF5B-RET fusion after cetuximab resistance that had never been reported in CRC.

Case Presentation

A 63-year-old male, who suffered from hematochezia and abdominal pain, was diagnosed with stage (pT3N1M1) colorectal carcinoma with liver metastases in June 2020. Since that the patient presented with intestinal bleeding, surgical resection was performed to alleviate the symptom. Endoscopic resection revealed two lesions on the surface of the colorectal, named A and B, and only tumor A was removed (Fig. 1). To search for an efficient therapeutic strategy, genomic DNA from formalin-fixed paraffin-embedded (FFPE) sample of tumor A and circulating tumor DNA (ctDNA) from plasma were subjected to targeted next-generation sequencing (NGS) of 425 cancer-related genes (Nanjing Geneseeq Technology Inc.) (Fig. 2A). Their genetic alterations were compared in Table 1. To our surprise, the mutation pattern was completely different between plasma and tumor A. Based on the KRAS G12D with a mutation allelic frequency (MAF) of 41.9% identified in tumor A, the patient was administrated with the XELOX plus bevacizumab (oxaliplatin 130 mg/m² day 1, capecitabine 1500 mg/m² twice daily for 14 days, bevacizumab 7.5 mg/kg day 1) for every 3 weeks as first-line treatment. The patient achieved an initial partial response (PR) with sustained response ongoing for 11 months. In January 2021, the tumor was evaluated PD, which led to second-line chemotherapy involving irinotecan (180 mg/ m² day 1), raltitrexed (3 mg/ m² day 1) and bevacizumab (5 mg/kg day 1) for every 2 weeks. However, the size of liver lesion increased 35% compared to baseline, which indicated a PD (Fig. 2B).

In April 2021, the plasma and tumor B were subjected to NGS, which identified four same mutation types without any KRAS mutations (Table 1). Compared genomic alterations between the tumors A and B, the mutation landscapes were completely different. Additional, Immunohistochemistry (IHC) staining showed significant differences between the two tumors (Supplementary Figure S1), consistent with the determination of MPCC. Based on the wild-type KRAS, the patient was subjected to the treatment of irinotecan (180 mg/ m² day 1), raltitrexed (3 mg/ m² day 1), plus cetuximab (500 mg/ m² day 1), a monoclonal antibody that blocks the epidermal growth factor receptor (EGFR), for every 2 weeks in April 2021. Plasma ctDNA sequencing and CT scan were performed every two weeks and two months, respectively (Fig. 2B and C). Two months later, the size of liver metastases decreased by 75% compared to the results of last detection and ctDNA abundance quickly decreased to <1% (Fig. 2B and D). Moreover, the tumor markers in CRC, carcinoembryonic antigen (CEA) and carbohydrate antigen 19–9 (CA19-9), also dramatically slipped to the normal level (Fig. 2C), which indicated a PR. Stable disease (SD) was observed in August 2021, with a 4% decrease in the size of liver metastases compared to last detection with no obvious increase in the level of CEA and CA19-9 (Fig. 2B and C), however, the allelic frequencies (AFs) of ctDNA alterations in plasma samples were dramatically elevated (Fig. 2D and Table 1). One month later, the tumor size increased by 30% compared to last month with an obvious increase in CEA and CA19-9 (Fig. 2B and C), which indicated a PD. Due to the occurrence of KIF5B-RET fusion (MAF = 18.5%), we recommended the use of pralsetinib, a selective RET inhibitor, but the patient refused. Then, the fourth-line chemotherapy with XELOX and bevacizumab was treated. Two months later, the tumor size increased by 21% (Fig. 2B). Unfortunately, he died of hepatic failure later.

Discussion

MPCC was first discovered by Warren S in 1941 (6). Despite its rareness, the occurrence rate of MPCC is an upward trend (2). Due to the lack of understanding of MPCC and the limitations of diagnosis techniques, it is always challenging to distinguish whether it is multiple primary cancer or tumor metastasis. The emergence of NGS has already changed the ways that we performed cancer studies, and been widely used in the diagnosis of multiple primary cancers (7, 8). In the case, the separate tumor lesions, tumor A and B, were firstly misdiagnosed as a primary lesion with its metastasis that shared similar features. Thus, only tumor A and plasma samples were subjected to targeted NGS. To our surprise, the mutation patterns were completely different between them. Then, targeted NGS was performed in tumor B and demonstrated that these two tumor lesions, A and B, didn't share any mutations. We were able to confirmed that tumor A and B were independent primary tumors inferred by genetic profiling. Of note, the molecular variations depicted by NGS facilitated the diagnosis of multiple primary tumors. We also supposed that tumor B may contribute to the liver metastases. However, the patient refused the liver biopsy. The follow-up NGS investigation confirmed the above conjecture due to a good consistency between tumor B and plasma ctDNA.
Surgical intervention has long been the ideal choices for cancer patients (9), but not for patients with metastatic lesions. Our patient presented with intestinal bleeding, we had to perform surgery to alleviate the symptom. For those patients who can't receive surgery, radiotherapy and chemotheraphy are the leading strategies for controlling disease (10). Targeted therapy is also a new optional approach that successfully prolonged overall survival for CRC patients (1). The first targeted agent for CRC approved by the Food and Drug Administration (FDA) was cetuximab, a monoclonal antibody that blocks EGFR, in 2004 (11). The response to anti-EGFR therapy is linked to the mutational status of downstream signaling molecules of the EGFR pathway (ie, KRAS, NRAS, PIK3CA, and BRAF). Only patients with a KRAS wild-type are likely to respond to the therapy (12). In our case, the tumor B and ctDNA showed a KRAS wild-type and the tumor A with KRAS G12D had been resected. Therefore, the cetuximab was administrated and the patient benefited from it with shrinking tumor in the liver, decreased AF of ctDNA and serum tumor markers (CEA and CA19-9). However, the patient presented drug resistance via NGS firstly, followed by serum tumor markers and CT image. As previous report, the serial ctDNA profiling can forecast disease progression earlier than CT scanning (13). Additionally, longitudinal ctDNA monitoring can provide a more accurate landscape of tumor that may improve personalized treatment decision-making (14). To the best of our knowledge, it was the first time to discover KIF5B-RET fusion when a CRC patient showed resistance to cetuximab. The emerging RET fusion variant is a crucial driver gene for drug resistance in multiple progressive cancers, like non-small cell lung cancer. Zhu et al. reported the emergence of the KIF5B-RET fusion gene may be a cause of acquired resistance to EGFR-TKIs in EGFR-mutant lung adenocarcinomas (15). Hence, we propose that this KIF5B-RET fusion gene could be a novel cause of acquired resistance to cetuximab in KRAS wild-type CRCs. However, the patient refused the pralsetinib, a selective RET inhibitor. The limitation of the single-case presentation in this study should be noted. The KIF5B-RET fusion in the case might be a potential resistance mechanism of cetuximab, however, additional pre-clinical studies and clinical evidence are needed.

Conclusion

In summary, we reported a rare case of a 63-year-old male patient with MPCC diagnosed by genetic profiling. The patient was treated with cetuximab based on a wild-type KRAS and developed novel mutations including KIF5B-RET fusion, which provides possible resistance mechanism to anti-EGFR therapy. This case highlights the necessity of using genetic testing for primary tumor diagnosis and the importance of longitudinal ctDNA profiling, which may trigger the development of effective therapeutic strategies.

Declarations

Date availability

All datasets generated for this study are included in the manuscript.

Declarations of interest

Authors Evenki Pan and Peng Yang are employed by Nanjing Geneseeq Technology Inc. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Ethics Approval and Consent for Publication

This research was approved by the Ethics Committee of The Second Hospital of Dalian Medical University. Written informed consent to publish the clinical details and images were obtained from the patient.

Author contributions

All authors contributed to data analysis and drafting or revising of the manuscript. All authors agreed on the journal to which the article is submitted, provided final approval of the manuscript version to be published, and agreed to be accountable for all aspects of the study.

References


**Table**

Table 1. The allele frequencies of genetic alterations detected by targeted NGS in the tumor A and B, and serial plasma ctDNA.
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<td>APC</td>
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<td>APC</td>
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<td>APC</td>
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<tr>
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<td>5.2%</td>
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FFPE: formalin-fixed, paraffin-embedded; "-": not detected; ctDNA: circulating tumor DNA.

**Figures**
Figure 1

Schematic diagram of the use of targeted next-generation sequencing (NGS) for the finding of double primary colorectal cancer, corresponding treatment and efficacy.
Figure 2

Sequence of the patients’ multiline treatments and Computed tomography (CT) images, the levels of colorectal cancer biomarkers, the allele frequencies (AFs) of ctDNA alterations during the treatments. (A) Timeline of multiline therapies received by the patient. (B) CT images of liver metastases during the treatments. Lesions are indicated by the red cycles. The number represents the change of the size of liver metastases from the previous image. (C) The levels of the colorectal cancer biomarkers carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) are shown by the blue and black lines, respectively. The four background colors represent every line treatment. (D) The AFs of ctDNA alterations are shown during the cetuximab treatment.

Supplementary Files

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- SupplementaryFigureS1.jpg