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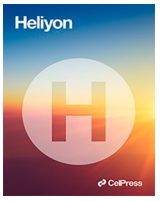

Case report

Rare *BRAF* gene fusions in metastatic early-onset colon cancer: A case report

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ABSTRACT

Gene fusions offer new therapeutic options for patients with metastatic colon cancer (CC). *BRAF* gene fusions are infrequent somatic mutations found in CC with prognostic and promising targeted therapies. CC diagnosed before the age of 50 was regarded as early-onset CC (EOCC). The incidence of EOCC is increasing, yet there is a clear unmet need to improve the management of EOCC. Herein, we selectively reported a case of metastatic EOCC with rare *BRAF* gene fusions. The right-sided tumors were radically resected. Next-generation sequencing (NGS) was performed on formalin-fixed paraffin-embedded tissues to eliminate gene variations. Histologically, the colonic hepatic flexure showed focal mucinous adenocarcinoma changes along with high-grade intraepithelial neoplasia. The results of histopathological examination belonged to pT1bN1bM0 IIIA stage. Targeted DNA sequencing revealed *AGAP3::BRAF* (A10;B9) fusion and *BRAF::AGAP3* (B8;A11) fusion were simultaneously detected in this case. Microsatellite instability-high (MSI-H) and *RAS/BRAF* ^{V600E} mutations were not detected. During a limited 1.5-year follow-up period, neither a confirmed local recurrence nor a distant organ metastasis occurred in this case. We propose that *BRAF* fusion variations can occur in metastatic EOCC.

1. Introduction

Colon cancer (CC) is one of the most frequent and deadly tumors worldwide [1]. CC is a malignant development that begins in the inner lining of the colon and can spread to deeper layers of the intestinal wall [2]. CC has a very complicated and poorly understood pathogenesis. CC is currently thought to be highly related to heredity, nutrition, inflammatory bowel disease, and other variables [3]. When cancerous cells manifest distant metastases or local recurrences, the prognosis is still dismal despite advancements in CC screening, diagnosis, chemotherapy, and targeted therapy [4]. CC diagnosed before the age of 50 was regarded as early-onset CC (EOCC) [5]. Even if EOCC is increasing, there is still a pressing need to enhance EOCC management.

Biomarker research has lagged, possibly due to lower pharma interest, difficulties with tissue availability, and the need for complex platforms for most prognostic biomarkers [6]. The Royal Marsden Hospital (RMH) score, based on readily available blood tests and clinical features, has emerged as a prognostic biomarker in patients with advanced colorectal cancer [7]. The comprehensive meta-analysis, encompassing over a hundred thousand patients, revealed a negative association between a higher RMH score and survival in cancer patients [6]. Therefore, the RMH score's generalizability may provide certain guidance for the prognostic management of CC patients.

The serine/threonine kinase protein *BRAF* mediates the Ras-Raf-MEK-extracellular signal-regulated kinase (ERK) signaling

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pathway, which controls cell division and differentiation [8]. Two percent of individuals with metastatic colorectal cancer have non-V600 BRAF mutations [9]. The functional category of $^{Non\text{-}V600}$*BRAF* mutations has a significant impact on prognosis and treatment response. Oncogenic gene fusions caused by chromosomal rearrangements are a significant clinical factor in the development of many malignancies. Therefore, quick and sensitive techniques are required to identify a variety of gene fusions in clinical specimens, which are frequently of low quality and quantity [10]. The use of next-generation sequencing (NGS) in routine clinical practice to find individuals with potentially useful mutations is growing. *BRAF* fusion genes provide an alternate way for activating mitogen-activated protein kinase (MAPK) signaling, they were first reported in pediatric gliomas [11]. Recently, *BRAF* fusion genes have been discovered in certain melanomas with *AGAP3::BRAF* fusion mutations [12].

Immune and BRAF-targeted therapies have changed the therapeutic scenario of advanced melanoma, turning the clinical decision-making a challenging task. The anti-melanoma efficacy of immune checkpoint inhibitors (ICIs) has increasing emerged during the last few years [13]. However, ICIs are related to various immune-related adverse events (irAEs), including hearing loss, neurotoxicity, and liver toxicity [14–16]. Acquisition of an *AGAP3::BRAF* fusion was linked to clinical resistance to vemurafenib in melanoma with a *BRAF* ^{V600E} mutation [17]. The detection of this *AGAP3::BRAF* fusion seems important for the choice of a targeted treatment since it has been described to confer resistance to BRAF-targeted therapies and EGFR-targeted in melanoma and colorectal cancer, respectively [18].

Therefore, we provide here the results of an extensive investigation of a metastatic CC from a 40-year-old patient who had rare *AGAP3::BRAF* fusion and *BRAF::AGAP3* fusion, expanding the molecular genetic spectrum of CC and providing novel treatment options for patients with unique subtypes of these features.

2. Case presentation

The 40-year-old male patient suffered from occasional diarrhea due to the consumption of raw and cold foods. A physical examination revealed occult blood in the stool. The serum level of carbohydrate antigen 72–4 was 34.7 U/mL (reference range: 0–6.9 U/mL), and other serum levels were normal. Among them, the albumin level was 45.22 g/L (reference range: 40–55 g/L) and the lactate dehydrogenase (LDH) level was 150 U/L (reference range: 120–250 U/L). The patient had a low RMH score (0). Colonoscopy showed that the colonic polyps had a soft texture and clear borders with the surrounding tissues. Colonic protrusions originated from the mucosal layer and had a slightly congested surface (Fig. 1A). The patient was initially diagnosed with adenomatous polyposis and underwent surgical resection. At a distance of 30 cm from the anus, approximately 1.2 × 0.8 × 0.6 cm wide basal polyp-like protrusions could be observed at the colonic hepatic flexure with a distinctly hyperemic and lobulated surface. The histopathological results of the hepatic flexure showed moderate dysplasia and mucinous changes (Fig. 1B). The patient underwent right hemicolectomy, ascending colon-transverse colon anastomosis, and regional lymph node resection. The original tumors on the ascending colon were completely excised (Fig. 1C and D). The histopathological results showed that the patient had two lymph node metastases around the

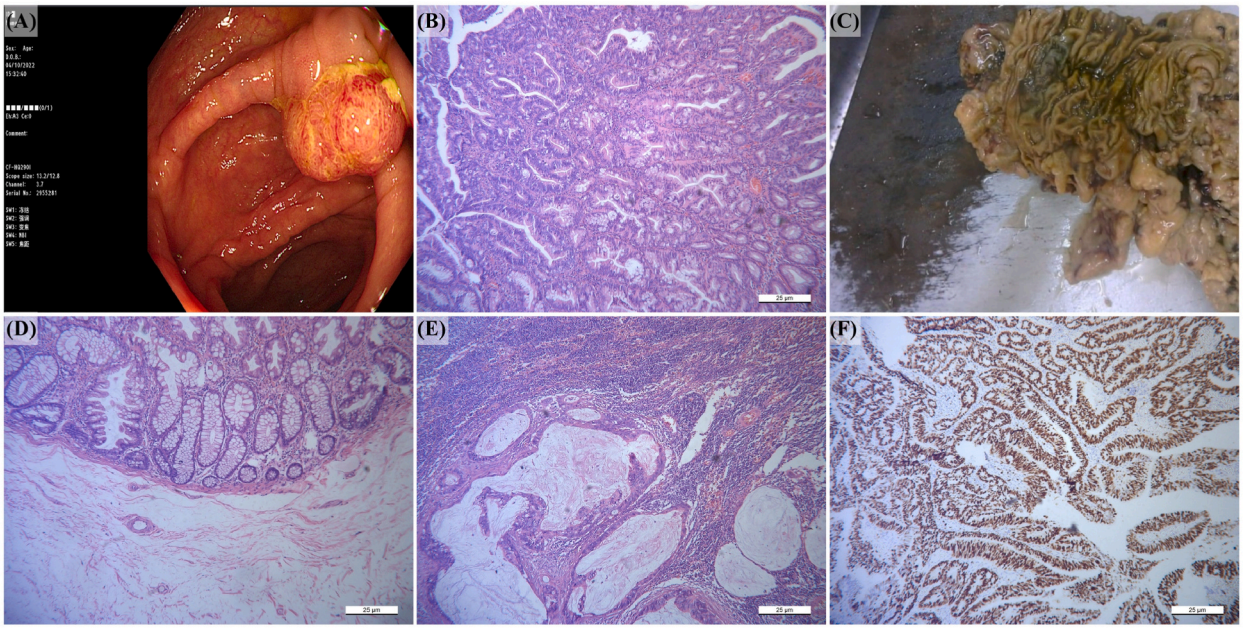

Fig. 1. Histological features of the metastatic EOCC patient. (A) Multiple polyps of the colon in patients undergoing colonoscopy. (B) The histopathological results of the colonic hepatic flexure. (C) Postoperative resection of the colon tumor. (D) The histopathological features of postoperative normal tissues. (E) H&E images of the metastatic lymph nodes around the colon. (F) Immunohistochemical result of Ki-67 expression in tumor cells.

intestine, and the American Joint Committee on Cancer pathological tumor-node-metastasis (pTNM) stage was pT1bN1bM0 IIIA (Fig. 1E). Immunohistochemistry staining showed that the patient was positive for MLH-1, PMS-2, MSH-2, MSH-6, and 70 % of Ki-67 expression (Fig. 1F).

Using NGS with the 769-gene panel, we detected an uncommon in-frame fusion of the exon 10 of *AGAP3* (7q36.1; NM_031946) with the exon 9 of *BRAF* (7q34; NM_004333) (Fig. 2A). On chromosome 7, the breakpoints were found at chr7:150828536 and chr7:140492743, in that order. In the meantime, we first detected an in-frame fusion between the exon 8 of *BRAF* and the exon 11 of *AGAP3* (Fig. 2B). The breakpoints on chromosome 7 were located at chr7:140492740 and chr7:150828524, respectively. In the tumor presented in this study, the number of *TP53* (17p13.1; NM_000546) copies was decreased (CN = 1.17), and the *TP53* gene c.844C>T mutation was detected, causing a synonymous change at p.R282W (52.04 %). This metastatic CC case was microsatellite stable (MSS), consistent with immunohistochemical staining results. The tumor mutation burden (TMB) was 4.87 Mut/Mb.

The patient's disease has remained stable to date. The timeline of the main events is outlined in Fig. 3. During a 1.5-year follow-up period, no confirmed local recurrence or distant organ metastases were found.

3. Discussion

The accumulation of genetic and epigenetic changes that transform a healthy glandular epithelium into invasive cancer is known as CC. In CC, genomic instability occurs through three pathways: chromosomal instability (CIN), MSI, and CpG island methylator phenotype [19]. The epidermal growth factor receptor (EGFR) signaling pathway is one of the most well-known CIN mechanisms. Similar to the effects of *KRAS* mutation, patients with *BRAF*-mutated CC do not respond to anti-EGFR monotherapy (panitumumab or cetuximab) and have shorter progression-free survival and overall survival than those with *BRAF* WT tumors [20]. It is well established that the *RAS/BRAF* mutations have a negative impact on the prognosis of metastatic CC and are predictive of response to targeted treatments. However, there have been few investigations in metastatic EOCC on the association between this mutational status and the disease's prognosis and recurrence patterns [21].

In our retrospective case series, a wide range of malignancies underwent extensive genomic profiling for hundreds of known cancer genes using NGS. *BRAF* fusion is a rather uncommon kind of *BRAF* mutation. We analyzed the NGS results of 318 colorectal cancer patients, *BRAF* mutation frequency was 6.92 % and *BRAF* fusion frequency was 0.31 %, which was consistent with the literature reports [22]. *BRAF* fusions rarely occur in the same gene arrangement, posing challenges for individualized therapy design. It is unclear how the diversity of fusion partners affects *BRAF's* oncogenic activity throughout tumor growth and treatment response.

The *AGAP3::BRAF* fusion has been documented in several tumor types, including melanoma [17], colorectal carcinoma [22], ovarian serous carcinoma [23], pancreatic acinar cell carcinoma [18], and gastrointestinal stromal tumor [24]. We simultaneously detected *AGAP3::BRAF* (A10;B9) (3.65 %) fusion and *BRAF::AGAP3* (B8;A11) (8.88 %) fusion in this case, which differed from exon9-exon9 and exon8-exon9 configurations previously described [25,26]. *AGAP3* is a critical component of the N-methyl--D-aspartate receptor-signaling complex, responsible for long-term potentiation in synapses [27]. An N-terminal GTPase-like domain, a pleckstrin homology domain, an ArfGAP domain, and multiple C-terminal ankryn repeat domains are all present in the encoded protein. The protein is primarily expressed naturally in the cerebral cortex, although colorectal carcinoma and adenoma have been observed to overexpress it [28]. While the 5′ fusion partner of *BRAF* fusion genes affects intracellular signaling and sensitivity to targeted therapies, Stangl et al., demonstrated that all examined *BRAF* fusions confer resistance to clinically meaningful EGFR

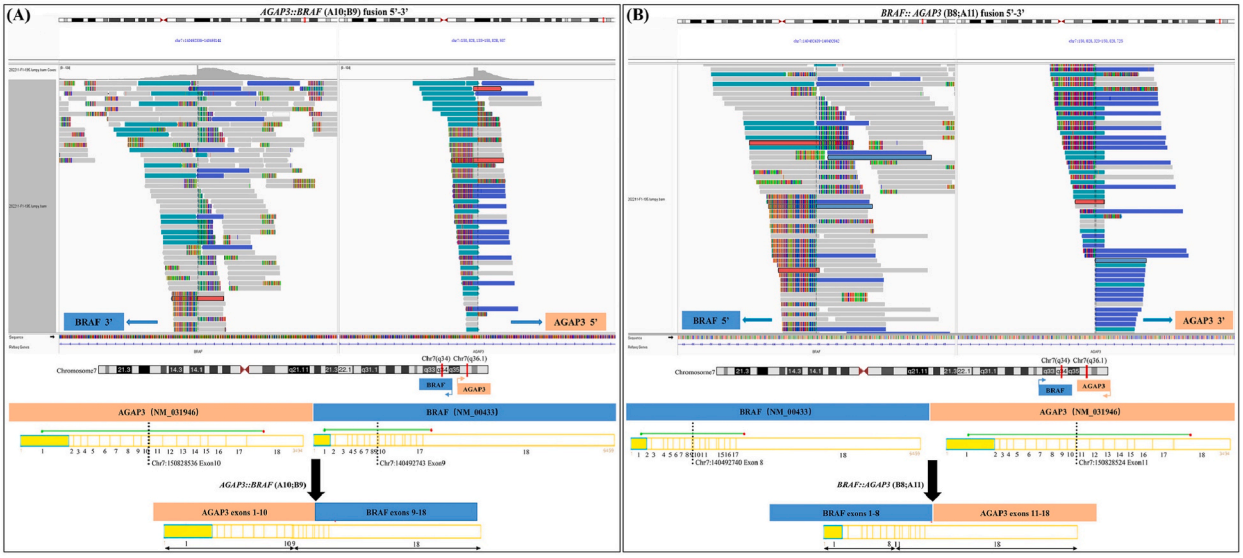

Fig. 2. An integrative genomics viewer snapshot of gene fusions by NGS. (A) In-frame fusion of *AGAP3* exon 10 and *BRAF* exon 9. (B) In-frame fusion between *BRAF* exon 8 and *AGAP3* exon 11.

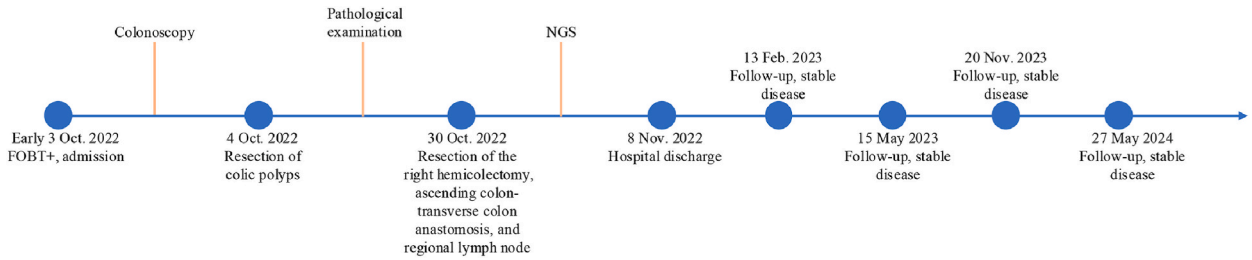

Fig. 3. Timeline of the main events. FOBT+: positive fecal occult blood test.

inhibition in colorectal carcinoma [22]. Therefore, *BRAF* fusions should be screened to exclude patients with metastatic CC from anti-EGFR treatment. In cancerous cells, MAPK signaling pathways are constitutively active and promote the development of resistance to MEK inhibition [29]. The MEK inhibitor, selumetinib (AZD6244, ARRY-142886) has been widely used to inhibit pERK1/2 phosphorylation in *BRAF* fusion mutated tumors [30]. Regretfully, there is currently no data on MEK inhibitors for patients with *BRAF* rearranged metastatic CC, which emphasizes the pressing need to generate proof in favor of this innovative treatment approach.

Better identification of patients with high gene fusion is an important issue from a therapeutic and medico-economic perspective. There are currently several techniques for identifying fusion genes or gene rearrangements. FISH analysis is not an optimal option for the detection of gene rearrangements [18]. NGS should be used instead, ideally with a suitable panel and an anchored multiplex PCR approach.

The RMH prognostic score includes albumin level, LDH level, and number of metastases [6]. Patients with a low RMH score (0–1) had a significantly longer median overall survival (OS) of 33 weeks compared to 15.7 weeks for patients with a high RMH score (2–3) [31]. This EOCC patient had a low RMH score. Therefore, we predict that the patient has a longer survival. Therefore, future research should aim to validate and refine RMH score, ensuring its optimal application in clinical practice and decision-making.

To our knowledge, the majority of earlier research identifying fusion genes in CC omitted information about clinical or histopathologic characteristics, particularly stage [26]. As opposed to previous studies, we found that fusion genes were present in pT1bN1bM0 IIIA CC. By making our dataset available, we want to help overcome the sample size constraints of individual investigations and advance our understanding of the potential therapeutic value of particular rare fusion genes.

4. Conclusion

We reported infrequent *AGAP3::BRAF* and *BRAF::AGAP3* gene fusions in metastatic CC. The diversity of partners is confirmed by the discovery of *AGAP3* as a rare fusion partner gene in the metastatic CC scene. These results emphasize the value of NGS in the identification and management of CC patients. NGS may be able to find more uncommon variants, give clinicians and CC patients more thorough mutational information, and assist in determining the best course of treatment. Future research involving more instances could contribute to our understanding of the clinical behavior and histological range of this fusion mutation in tumors. We will continue to conduct regular follow-up and disease monitoring in this case.

CRediT authorship contribution statement

Tingting Zhao: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Junting Yang:** Validation, Supervision, Software. **Meirong Wang:** Visualization, Software, Methodology. **Jie Liu:** Writing – review & editing.

Ethics statement

Written informed consent was obtained from the patient for the publication of any potentially identifiable images or data included in this article. The ethic approval number is 2024-359.

Guarantor statement

Tingting zhao confirms full responsibility for the content of this manuscript.

Data availability statement

Data will be made available on request.

Declaration of generative AI in scientific writing

Artificial intelligence has not been used in the writing process.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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