Case Reports in Oncology

Case Rep Oncol 2020;13:595–600
DOI: 10.1159/000507882
Published online: June 4, 2020

Case Report

KRAS and *BRAF* Concomitant Mutations in a Patient with Metastatic Colon Adenocarcinoma: An Interesting Case Report

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Keywords
Colorectal cancer · *KRAS* · *BRAF* · Therapeutic responses

Abstract
A 68-year-old female patient with tenesmus and blood in the stool was admitted to the S.G. Moscati Hospital of Taranto. Investigations revealed infiltrative mucinous colon adenocarcinoma accompanied by lymph node metastases. Following surgery and adjuvant chemotherapy, computed tomography (CT) and carcinoembryonic antigen screening were negative. Two years later, CT demonstrated a liver lesion. Histologic and genetic analyses confirmed the diagnosis of metastatic colorectal cancer with the coexistence of *KRAS* and *BRAF* mutations in hepatic metastases and the presence of the *BRAF* V600E in the primary tumour. It is unclear whether the lack of response was due to *BRAF* mutations, but the data suggest that mutated *BRAF* confers resistance to anti-epidermal growth factor receptor therapy. In our patient, *BRAF* mutation turned out to be a negative prognostic factor, and it may have been the cause of clinical implications for disease progression and therapeutic responses.

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and cause of death among men and women. At stage IV of CRC, liver metastases occur in 20–30% of patients, whereas peritoneal and lung metastases occur in 10–15% and 10–25% of patients, respectively, and other non-rectal or non-colon metastases occur rarely [1]. CRC progresses through

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a series of well-defined steps associated with specific genetic and epigenetic alterations in various oncogenes and tumour suppressor genes [2]. Approximately 50–60% of colorectal tumours are mutated (missense mutation) in the *KRAS* gene, and approximately 5–10% of tumours show mutation in the *BRAF* gene (missense mutation). These mutations in KRAS and BRAF oncoproteins activate signalling cascades that mediate cellular responses such as cell proliferation, apoptosis, adhesion, invasion and angiogenesis. Mutations in *RAS* genes (*KRAS* exons 2, 3 and 4 and *NRAS* exons 2, 3 and 4) located downstream from epidermal growth factor receptor (EGFR) within this pathway lead to its activation even if EGFR is blocked [2, 3]. Although the *BRAF* gene is located downstream of *KRAS*, the activating V600E *BRAF* mutation is not considered a predictive biomarker for resistance to anti-EGFR antibody therapy. However, mutations in this gene have been suggested to be strong prognostic markers of poor prognosis in CRC patients [4].

The EGFR signalling pathway becomes constitutively active in these tumours, so that now the strategy of drug development is moving towards skilled aiming at the RAS pathway [5]. Molecular screening, such as for Val600Lys in the *BRAF* gene, is crucial for the care of CRC patients, and it might significantly improve the cost-effectiveness and important consequences regarding treatment. In this study, we report a case of metastatic CRC with coexistent *KRAS* and *BRAF* mutations in a 68-year-old woman affected by advanced adenocarcinoma of the rectum and liver metastases. Concomitant *KRAS* and *BRAF* mutation in CRCs is rare, occurring in less than 0.001% of cases [6], but this event appears to be associated with the presence of *BRAF* mutation in the primary tumour and with a more aggressive outcome, as in this case. These are two activators in the protooncogenes that induce a functional loss of tumour suppressor genes. *RAF* mutations in CRC are mostly V600E amino acid substitutions, although various other mutations at codon 600 or neighbouring positions within the kinase domain are documented, too. Structural studies of RAF proteins have identified the valine at position 600 as a crucial site within the conserved kinase domain, which is required for BRAF to maintain an inactive conformation in the absence of KRAS-BRAF interaction [7]. Mechanistically, mutations at this site likely render *BRAF* constitutively active, bypassing dimerization with BRAF or RAF1, which is normally a prerequisite for activation. Consequently, the V600E mutation is strongly activating, resulting in constitutive MEK binding, phosphorylation and therefore BRAF signal transduction. *BRAF* amplification and/or loss of heterozygosity have infrequently been detected in CRC [8]. The significance of these *BRAF* genomic imbalances is unclear; however, *BRAF* copy number gains have been implicated in drug resistance of CRC. Metastatic CRC with concomitant *RAS* + *BRAF* mutations should be assigned to a separate arm in clinical trials to evaluate the role of novel therapeutics for this deadly disease.

Case Presentation

Patient and Treatments

In July 2015, a 68-year-old female patient with rectal tenesmus and blood in the stool underwent colonoscopy at the S.G. Moscati Hospital of Taranto. The examination revealed a fungating and bleeding stenotic mass. Histologic analysis of a biopsy from this mass supported the diagnosis of adenocarcinoma. A total body computed tomography (CT) scan showed a thickening of the descending colon wall and the presence of pericentromeric lymph nodes in the pericolic fat tissue. After a few days, the patient was admitted to the Surgery Department, SS Annunziata Hospital of Taranto, and colectomy and splenectomy were subsequently carried out.

The final histologic diagnosis was infiltrative mucinous adenocarcinoma, with metastases in 4 out of 17 resected lymph nodes, but no pathological aspects were observed in the spleen (pT3pN2aMx G2). After surgery, the patient received adjuvant chemotherapy with 12 cycles of FOLFOX regimen (fluorouracil + folinic acid + oxaliplatin). Postoperative CT scan examination was negative, and carcinoembryonic antigen and gastrointestinal cancer antigen levels were within the normal range. In February 2017, a total body CT scan evidenced a suspicious liver lesion between segments VI and VII and a liver biopsy was carried out. Histologic analysis confirmed the colic origin of the metastasis by positivity for CK20 and CDX2. Metronomic treatment with capecitabine was started.

After obtaining informed consent from the patient, the immunohistochemical EGFR expression profile was investigated by anti-EGFR monoclonal antibody according to the manufacturer's descriptions. Paraffin-embedded tissue sections were collected on microscopic slides. Molecular assessment of the *KRAS* and *BRAF* genes on the liver biopsy and the primary tumour was performed at the Pathology Department, SS Annunziata Hospital of Taranto. Haematoxylin and eosin slides were reviewed by the pathologist to confirm the diagnosis and select the best representative area of the tumour for DNA extraction.

DNA Extraction

Tree 10-μm-thick unstained sections were cut from the previously selected paraffin blocks. The slides were deparaffinized with xylene. The neoplastic tissue sample was obtained by manual macrodissection. DNA was extracted with a QIAcube Instrument (QIAGEN). The DNA concentration was measured on a QUBIT instrument (Thermo Fisher Scientific, Waltham, MA, USA), and the minimum DNA concentration for the experiments was set to 2 ng/μL.

Analysis of KRAS *and* BRAF *Mutations*

Mutation analysis was conducted by pyrosequencing in the coding sequence of the *KRAS* gene (exon 2 and exon 3) and the *BRAF* gene (exon 15) using the *therascreen*® *KRAS* and *BRAF* Pyro Kits, respectively.

Results

In hepatic tissue, the identified alterations in the *KRAS* and *BRAF* genes were a mutation in codon 12 (c.35G>A p.G12D) of exon 2 and a missense nucleotide base change in codon 600 (c.1799T>A GTG to GAG p.V600E) of exon 15, respectively, while in the primary tumor there was only *BRAF* mutation.

The patient's case was discussed by a multidisciplinary tumour board. The board's recommendation was for upfront systemic treatment with 12 cycles of FOLFIRI (5FU) + Avastin (bevacizumab) and a next evaluation pending determination of a response to chemotherapy (January 2018). A total body CT in July 2018 showed pulmonary microlesions, increased hepatic lesions and deep venous thrombosis of the left gonadal vein. The patient's case was discussed by the multidisciplinary tumour board, and in August the patient received chemotherapy with Stivarga (regorafenib) 40 mg, starting with 2 tablets/day for 1 week and then 3 tablets/day for 3 weeks. The course of treatment was complicated by side effects: asthenia, lack of appetite and resistant shoulder pain. A total body CT in November 2018 showed increased pulmonary, hepatic and renal lesions. In December 2018, the patient received third-line therapy with FOLFOX at low doses and oral Lonsurf (trifluridine/tipiracil). After 3 weeks, clinical improvement was reported with lack of appetite and good pain control. A total body CT in July 2019 showed only increased pulmonary lesions.

Discussion

This rare case of metastatic CRC with coexistent *KRAS* and *BRAF* mutations had several unusual features, including rapid progression of disease. It has been shown that patients who have both *KRAS* and *BRAF* mutations tend to have an adverse outcome [9–12]. No possible mechanism underlying coexistent *KRAS* and *BRAF* mutation is known. Furthermore, it is unclear whether these tumours have a different biology and natural history than single *KRAS* or *BRAF* mutant tumours, or which of the two mutations is the dominant oncogene driving tumour proliferation [10–14]. It is known that most *BRAF* mutations identified in CRC are V600E, which is a class I mutation. The valine at codon 600 lies within the kinase domain and is required for BRAF to maintain an inactive status in the absence of KRAS-BRAF interaction. The V600E mutation results in amino acid substitution from a valine to a glutamic acid, leading to 130- to 700-fold increased BRAF kinase activity compared with wild-type *BRAF* [15]. In metastatic CRC, patients with *BRAF* V600E mutation are not likely responding to anti-EGFR therapy, and they have decreased survival compared to patients with wild-type *BRAF* [16]. Jones et al. [15] have reported that non-V600E *BRAF* mutant metastatic CRC represents a clinically distinct molecular subtype, associated with significantly longer overall survival compared to that of metastatic CRC patients with a *BRAF* V600E mutation. A possible mechanism to be considered is the presence of a biclonal population of cancer cells, with a clone harbouring a *KRAS* mutation and the other clone harbouring a *BRAF* mutation.

The impact of *BRAF* mutations has also been retrospectively evaluated on tissue from completed prospective trials. MRC (Medical Research Council) COIN was the largest trial that studied the effect of the addition of anti-EGFR treatment (cetuximab) to a chemotherapy regimen of fluoropyrimidine in metastatic CRC [17]. The effect of cetuximab was further analysed for the presence/absence of *KRAS, NRAS* and *BRAF* mutations. The data showed that the addition of anti-EGFR drugs to standard chemotherapy for *BRAF* mutant metastatic CRC is associated with worse outcomes. In fact, the median overall survival was shorter with *BRAF* mutant CRC (8.8 months) than with *BRAF* and *KRAS* wild-type tumours (17.5 months) [11–14]. Bevacizumab is a drug approved by the FDA and used as an inhibitor of *BRAF* V600E in *BRAF* mutant melanoma. It has also been tested in *BRAF* mutant CRC but failed to show any clinical advantage and antitumour activity [18]. When bevacizumab blocks *BRAF* activity and cuts off signalling within the MAPK pathway, this kicks on a feedback mechanism leading to upregulation of upstream EGFR, once again driving signalling through the MAPK pathway upon which these tumours are so dependent. Thus, the optimal management of metastatic CRC harbouring concomitant *KRAS* and *BRAF* mutation is still unknown.

Conclusion

In standard clinical management of CRC, *KRAS* mutation serves as a predictive biomarker for the selection of patients eligible for anti-EGFR therapy, with a benefit recorded only for *RAS* wild-type tumours. As reported, concomitant *KRAS* and *BRAF* mutation is associated with more severe disease, and this emphasizes the importance of obtaining baseline testing of these mutations as a standard of care in the clinical management of metastatic CRC patients. Furthermore, this distinct and highly aggressive subset of tumours should be assigned to a separate arm in clinical trials to evaluate novel therapeutic approaches.

Statement of Ethics

All procedures were conducted according to the principles expressed in the Declaration of Helsinki and the Guideline for Good Clinical Practice. The patient provided written informed consent to participate, as well as for the publication of any relevant clinical information for scientific purposes.

Disclosure Statement

The authors have no conflicts of interest to declare.

Funding Sources

This research did not receive any financial support from funding agencies in the public, commercial or not-for-profit sectors.

Author Contributions

S.P. participated in the clinical diagnosis and the management of the patient. M.P. performed the histologic analysis. G.S. participated in the management of the patient. P.L.S. participated in the clinical diagnosis. C.C. performed the molecular genetic study and variant identification and drafted the manuscript. A.R. participated in molecular analysis and in the drafting of the manuscript. G.D. revised the work. All authors contributed to critical discussion and approved the final version of the manuscript.

Availability of Data and Materials

DNA from the patient and the original pyrosequencing are available upon request.

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