Case Report

# Concomitant *KRAS* and *BRAF* mutations in colorectal cancer

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**Abstract:** *BRAF* and *KRAS* are two key oncogenes in the RAS/RAF/MEK/MAP-kinase signaling pathway. While previously considered mutually exclusive, concomitant mutations in both *KRAS* and *BRAF* genes have been identified in colorectal cancer (CRC). The clinical outcome of these patients remains undetermined. We present the clinical course of two patients with CRC harboring mutations at codon 12 of *KRAS* and *BRAF* non-V600E mutations. More research is needed to determine the clinical-pathological effect of these simultaneous mutations of *KRAS* and *BRAF* in CRC on disease course and treatment outcome.

**Keywords:** *BRAF*; *KRAS*; colorectal cancer (CRC)

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## Introduction

Somatic mutations involving the GTP-ase RAS protein family and its downstream serine-threonine kinase BRAF lead to loss of cell cycle regulation at key checkpoints and are the main driver mutations for colorectal carcinogenesis (1). The reported incidence of *RAS* (including *KRAS* and *NRAS*) mutations in colorectal cancer (CRC) is about 53% (2), while *BRAF* is mutated less frequently at a rate of 10% (3). The most common point mutation in *KRAS* involves an amino acid substitution at codons 12 or 13 (G12D, G12V and G13D) in exon 2, but codons 59 and 61 in exon 3 and codons 117 and 146 in exon 4 may also be affected. Approximately 80% of mutation in *BRAF* has been identified as gain-of-function mutation in exon 15 leading to the substitution of glutamine for valine at codon 600 (V600E), which is more likely to be found in older women and associated with poorly differentiated tumors in the proximal (right-sided) colon (4). In addition, *BRAF* V600E mutation has been identified in 'mismatch repair deficient tumor cells' that are more prone to microsatellite instability due to epigenetic changes of the *MLH1* gene (5).

Mutation in either *RAS* or *BRAF* genes leads to resistance to anti-EGFR (epidermal growth factor receptor) therapies in patients with metastatic CRC, and is frequently associated with a poor outcome. Mutations of *RAS* and *BRAF* are usually mutually exclusive (6). The concomitant *RAS* and *BRAF* mutations are rarely identified, but are more frequently found in patients with microsatellite stable CRC (7-12). However, the detection rate for each mutation depends on the technique used, suggesting that the true incidence of concomitant *RAS* and *BRAF* mutant CRC might be higher than previously thought. The availability of panel gene sequencing modalities by next generation sequencing (NGS) could help identify more simultaneous mutations in genes involved in the RAS/RAF/MEK/MAP-kinase cascade. The clinical outcome in CRC harboring coexistence of *RAS* and *BRAF* mutations remains unclear. Herein, we report two cases with coexistence of *RAS* and *BRAF* mutations and their clinical courses.

## Case presentation

### *Case 1*

The patient was a 67-year-old African American female

who presented for evaluation of abdominal bloating and dyspnea in December of 2016. A computed tomography (CT) scan showed ascites and bilateral adnexal masses with solid and cystic components (up to 14 cm × 8 cm × 10 cm in size), as well as asymmetric rectal wall thickening. On subsequent colonoscopy, there was a non-obstructing rectal mass superior to the dentate line; biopsy of rectal mass showed a well-differentiated adenocarcinoma arising in a tubulovillous adenoma. Evaluation by Gynecologic Oncology suggested the bilateral adnexal masses were likely ovarian metastases from rectal cancer. Cancer antigen-125 level was elevated at 107.8 units/mL, but other tumor markers (carcinoembryonic antigen, cancer antigens 19-9 and 15-3) were within normal limits. A portion of the rectal tumor was submitted to a reference laboratory for molecular profiling of the *BRAF*, *HRAS*, *KRAS*, and *NRAS* genes by targeted NGS. Specifically, exons 11 and 15 of the *BRAF* gene, exons 2 and 3 of the *HRAS* gene, exons 2 through 4 of the *NRAS* gene, and exons 2 through 4 of the *KRAS* gene. Sequencing of the targeted genes identified co-existing mutations in the *BRAF* and *KRAS* genes. In the *KRAS* gene, there was a c.35G>T [p.G12V (Gly12Val)], leading to a missense mutation from a glycine (G) to a valine (V) in exon 2 codon 12. In the *BRAF* gene, there was a c.1396G>C [p.G466R (Gly466Arg)] mutation, resulting in glycine to arginine (R) change in the amino acid at exon 11 codon 466. Additionally, immunohistochemical study of tumor sample showed intact nuclear expression of MLH-1, MSH-2, MSH-6, and PMS2 protein, indicating intact DNA mismatch repair enzyme protein expression.

The patient's case was discussed at multi-disciplinary tumor board; the board's recommendation was for upfront systemic treatment followed by salvage surgery evaluation pending determination of response to chemotherapy. She started first-line chemotherapy with folinic acid, 5-fluorouracil and oxaliplatin (FOLFOX) from March 2017. A repeat CT scan in May 2017 showed minimal decrease in sizes of pelvic masses with continued evidence of peritoneal carcinomatosis. Treatment course was complicated by worsening ascites requiring two large-volume paracenteses (June 2017 and January 2018, no malignant cells found), and deep vein thrombosis of left lower extremity in July 2017. She tolerated FOLFOX well except grade 1 peripheral neuropathy, and completed six months of treatment; end-of-treatment CT scan in November 2017 showed stable disease. In March 2018, she underwent extensive surgical resection including abdominoperineal resection, greater omentectomy, total colectomy and peritonectomy. Intraoperatively, mitomycin C was administered over 90 minutes as hyperthermic intraperitoneal chemotherapy (HIPEC). Pathologic examination of the resection specimen showed moderately-differentiated rectal adenocarcinoma with invasion into muscularis propria. There was no lymphovascular or perineural invasion seen, and surgical margins were clear by 4 mm. None of forty-four regional lymph nodes examined were positive for metastatic tumor. Interestingly, the pelvic mass was most consistent with granulosa cell tumor of ovarian origin, and there were also foci of granulosa cell tumor involving the sigmoid colon and terminal ileum. Peritonectomy samples were negative for malignancy. Final staging of the primary rectal cancer was T2N0M0 (stage I). She has been under observation after surgery without evidence of disease.

### *Case 2*

A 60-year-old Asian male presented with bright red blood per rectum in 2014, and rectosigmoid adenocarcinoma found on colonoscopy. He underwent low anterior resection in October of 2014 with seven of seventeen lymph nodes positive for metastatic adenocarcinoma. At the time of surgery, he was staged as stage IIIc, pT3N2bM0. He did not receive adjuvant chemotherapy due to insurance issues. A PET-CT scan in April 2015 showed hypermetabolic activity at the surgical site and in an enlarging left upper lung nodule concerning for local disease recurrence with metastases. He received chemotherapy with FOLFOX from May to December 2015. Despite treatment, the left lung lesion increased from 1.8 cm × 1.4 cm on imaging, with left paratracheal and bilateral hilar lymphadenopathy also seen. The biopsy of left lung mass in December 2015 confirmed metastatic adenocarcinoma of colonic primary. He underwent video assisted thoracoscopic left upper lobe wedge resection and mediastinal lymph node dissection in February 2016.

The specimens from the resected pulmonary nodule were sent to a reference laboratory for NGS, demonstrating a *KRAS* mutation c.34G>A [p.G12S (Gly12Ser)] and *BRAF* mutation c.1390G>A [p.G464R (Gly464Arg)]. There was intact nuclear expression of MLH-1, MSH-2, MSH-6 and PMS-2 protein, consistent with microsatellite stability.

He received a course of post-operative treatment with bevacizumab plus folinic acid, 5-fluorouracil and irinotecan (FOLFIRI) from April to November 2016. In March 2017, he developed recurrent disease in sigmoid colon and retroperitoneal lymph nodes noted in PET-CT scan,

 

and confirmed by colonoscopic biopsy. In the subsequent 18 months, he failed FOLFIRI then bevacizumab plus folinic acid, 5-fluorouracil, oxaliplatin and irinotecan (FOLFOXIRI). He started regorafenib in September 2018.

## Discussion

It remains unclear about the impact of dual *KRAS* and *BRAF* mutations on overall or progression-free survival of CRC. Guglielmini *et al.* reported the clinical courses of three cases of CRC with concomitant *KRAS* and *BRAF* mutations (11). The two patients with *KRAS* G13D and *BRAF* V600E mutations presented with synchronous metastases (one with rectal primary); both were refractory to first-line chemotherapy with either FOLFOX or FOLFIRI, and died 6 to 12 months after initial diagnosis. The third patient with stage III right colon cancer and mutations of *KRAS* G12V and *BRAF* V600E achieved free of disease 6 months after surgical resection and adjuvant chemotherapy with FOLFOX. Vittal *et al.* reported a 29-year-old patient with rectal cancer, synchronous liver metastasis and mutations in *KRAS* G12D and *BRAF* V600E; this patient did not respond to first-line chemotherapy with FOLFOX and died three months following diagnosis (9).

Deshwar *et al.* retrospectively analyzed 820 CRC patients' primary or metatic liver tumor samples for *KRAS* and *BRAF* mutation; patients all received hepatic resection for liver metastases (10). They found the incidence of coexistence of *KRAS/BRAF* mutation was 0.5% (4/820). Of these cases, patient 1 (*KRAS* G13D and *BRAF* V600E), patient 2 (*KRAS* G12V and *BRAF* V600E), and patient 3 (*KRAS* G13D and *BRAF* D594N) succumbed to their disease within 485, 236 and 79 days respectively, after liver resection. Patient 4 (T4 primary, *KARS* G12S and *BRAF* G469S) was alive 416 days post hepatic resection. Patient 1 and 2 received pre-operative chemotherapy with FOLFOX, and patient 4 receive post-operative chemotherapy with FOLFOX.

Both of our patients had missense gain-of-function mutation in codon 12 of *KRAS* in their tumor samples. G12V in case 1 caused change of amino acid from glycine to valine. G12S in case 2 resulted in change of amino acid from glycine to serine. These two missense mutations have been shown to result in accumulation of constitutively GTP-bound KRAS, activation of RAS/RAF/MEK/ERK pathway signaling and increased tumor growth in CRC animal model as well as resistance to anti-EGFR therapies in patients with metastatic CRC (6,13).

*BRAF* mutations are categorized into three classes: high (I), intermediate (II), and impaired (III) kinase activity subtypes (14). Both patients in this report had *BRAF* non-V600E missense mutations in their tumor samples. G466R in first patient resulted in change of amino acid from glycine to arginine at codon 466. G466R (previously reported as G465R) has been shown to be a class III BRAF mutation leading to impaired BRAF kinase activity *in vitro* in CRC (15). G464R in second patient caused change of amino acid from glycine to arginine at codon 464. G464R (previously reported as G463R) is a class II *BRAF* mutation which confers an intermediate *BRAF* kinase activity and increased cell proliferation (16).

Most of *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 that of wild-type *BRAF* (17). In metastatic CRC, patients with BRAF V600E mutation are not likely responding to anti-EGFR therapy, and have decreased survival compared to patients with wild-type *BRAF* (12,18). Jones *et al.* have reported non-V600E *BRAF* mutant metastatic CRC represents a clinically distinct molecular subtype, which is associated with significantly longer overall survival compared to metastatic CRC patients with *BRAF* V600E mutation (15).

Our cases are similar to reports from others that patients with non-V600E *BRAF*- and *KRAS*-mutant CRC seem to have better outcome than patients with *BRAF* V600E mutant CRC. The first patient responded well to preoperative chemotherapy with FOLFOX for rectal adenocarcinoma with final pathological staging of T2N0 after surgery. Interestingly, she had a second primary malignancy with ovarian granulosa cell tumor, and received optimal surgery at the time of rectal resection. She has since been under surveillance without evidence of disease. Our second patient presented with locally advanced sigmoid colon adenocarcinoma, with disease recurrence shortly after surgery. He received first-line chemotherapy with FOLFOX then metastasectomy of pulmonary metastasis. He has received additional chemotherapy with irinotecan based regimens plus bevacizumab then regorafenib due to persistent disease, but with a rather indolent course lasting more than three years since development of metastatic disease. These cases also call into question how existing

 

chemotherapy regimens can best be combined or modified to achieve disease control in patients with metastatic CRC and coexisting mutations in *KRAS* and *BRAF*.

## Conclusions

With the increasing adoption of NGS panel testing, the incidence of coexisting *KRAS* and *BRAF* mutations in CRC is likely higher than previously realized. More investigation is needed to determine the effect of simultaneous *KRAS* and *BRAF* mutations on prognosis in CRC and responsiveness of these tumors to current cytotoxic and biologic therapies.

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## Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

*Informed Consent:* Written informed consent was obtained from the patient for publication of this manuscript and any accompanying images.

## References

1. Oikonomou E, Koustas E, Goulielmaki M, et al. BRAF vs RAS oncogenes: are mutations of the same pathway equal? Differential signalling and therapeutic implications. Oncotarget 2014;5:11752-77.
2. Sorich MJ, Wiese MD, Rowland A, et al. Extended RAS mutations and anti-EGFR monoclonal antibody survival benefit in metastatic colorectal cancer: a meta-analysis of randomized, controlled trials. Ann Oncol 2015;26:13-21.
3. van Brummelen EMJ, de Boer A, Beijnen JH, et al. BRAF Mutations as Predictive Biomarker for Response to Anti-EGFR Monoclonal Antibodies. Oncologist 2017;22:864-72.
4. Sanz-Garcia E, Argiles G, Elez E, et al. BRAF mutant colorectal cancer: prognosis, treatment, and new perspectives. Ann Oncol 2017;28:2648-57.
5. Clarke CN, Kopetz ES. BRAF mutant colorectal cancer as a distinct subset of colorectal cancer: clinical characteristics, clinical behavior, and response to targeted therapies. J Gastrointest Oncol 2015;6:660-7.
6. De Roock W, Claes B, Bernasconi D, et al. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol 2010;11:753-62.
7. Sahin IH, Kazmi SM, Yorio JT, et al. Rare Though Not Mutually Exclusive: A Report of Three Cases of Concomitant KRAS and BRAF Mutation and a Review of the Literature. J Cancer 2013;4:320-2.
8. Oliveira C, Velho S, Moutinho C, et al. KRAS and BRAF oncogenic mutations in MSS colorectal carcinoma progression. Oncogene 2007;26:158-63.
9. Vittal A, Middinti A, Kasi Loknath Kumar A. Are All Mutations the Same? A Rare Case Report of Coexisting Mutually Exclusive KRAS and BRAF Mutations in a Patient with Metastatic Colon Adenocarcinoma. Case Rep Oncol Med 2017;2017:2321052.
10. Deshwar A, Margonis GA, Andreatos N, et al. Double KRAS and BRAF Mutations in Surgically Treated Colorectal Cancer Liver Metastases: An International, Multi-institutional Case Series. Anticancer Res 2018;38:2891-5.
11. Guglielmini PF, Rossi M, Grosso F, et al. Double KRAS and BRAF mutations in colorectal cancer in a single oncologic department series. J Clin Oncol 2013;31:abstr e14657.
12. Van Cutsem E, Kohne CH, Lang I, et al. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. J Clin Oncol 2011;29:2011-9.
13. Modest DP, Camaj P, Heinemann V, et al. KRAS allel-specific activity of sunitinib in an isogenic disease model of colorectal cancer. J Cancer Res Clin Oncol 2013;139:953-61.
14. Dankner M, Rose AAN, Rajkumar S, et al. Classifying BRAF alterations in cancer: new rational therapeutic strategies for actionable mutations. Oncogene 2018;37:3183-99.
15. Jones JC, Renfro LA, Al-Shamsi HO, et al. Non-V600 BRAF Mutations Define a Clinically Distinct Molecular Subtype of Metastatic Colorectal Cancer. J Clin Oncol 2017;35:2624-30.
16. Houben R, Becker JC, Kappel A, et al. Constitutive activation of the Ras-Raf signaling pathway in metastatic melanoma is associated with poor prognosis. J Carcinog 2004;3:6.

 

17. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. Nature 2002;417:949-54.
18. Kawazoe A, Shitara K, Fukuoka S, et al. A retrospective observational study of clinicopathological features of KRAS, NRAS, BRAF and PIK3CA mutations in Japanese patients with metastatic colorectal cancer. BMC Cancer 2015;15:258.

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