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Double *KRAS* and *BRAF* Mutations in Surgically Treated Colorectal Cancer Liver Metastases: An International, Multi-institutional Case Series

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Abstract. *Background: While previously believed to be mutually exclusive, concomitant mutation of Kirsten rat sarcoma viral oncogene homolog (KRAS)- and V-raf murine sarcoma b-viral oncogene homolog B1 (BRAF)-mutated colorectal carcinoma (CRC), has been described in rare instances and been associated with advanced-stage disease. The present case series is the first to report on the implications of concurrent KRAS/BRAF mutations among surgically treated patients, and the largest set of patients with surgically treated colorectal liver metastasis (CRLM) and data on KRAS/BRAF mutational status thus far described. Case Series: We present cases from an international, multi-institutional cohort of patients that underwent hepatic resection for CRLM between 2000-2015 at seven tertiary centers. The incidence of KRAS/BRAF mutation in patients with CRLM was 0.5% (4/820). Of these cases, patient 1 (T2N1 primary, G13D/V600E), patient 2 (T3N1 primary, G12V/V600E) and patient 3 (T4N2 primary, G13D/D594N) succumbed to their disease within 485, 236 and 79 days respectively, post-hepatic resection. Patient 4 (T4 primary, G12S/G469S) was alive 416 days after hepatic resection. Conclusion: The present case series suggests that the incidence of concomitant KRAS/BRAF mutations in surgical cohorts may be higher than previously hypothesized, and associated with more variable survival outcomes than expected.*

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Approximately 135,000 new cases of colorectal carcinoma (CRC) are expected to be reported during the course of the current year in the United States (1). Unfortunately, around 65% of these patients will either present with or develop distant metastases, with the liver (40%) being the most common site of disease spread (2). In turn, several attempts have been made to assess the prognosis of patients with colorectal lives metastasis (CRLM) using clinicopathological risk factors. Nonetheless, a consistently accurate prognostic model for this patient population has yet to be developed (3). As such, recent studies have focused on the development and validation of biological markers that may provide more accurate prognostic forecasts in patients with CRLM (4).

Among the biomarkers studied so far, the presence of kirsten rat sarcoma viral oncogene homolog (*KRAS*) and V-Raf murine sarcoma viral oncogene homolog B1 (*BRAF*) mutations has been shown to have considerable prognostic value in both CRC and CRLM (5). Among patients with CRC, mutated *KRAS* is reported in up to 35-45%, while mutated *BRAF* has been found in approximately 10% of patients (6-10). The presence of *KRAS* and *BRAF* mutations has traditionally been considered mutually exclusive (11). However, recent case reports of metastatic CRC suggest that the concurrent presence of *KRAS* and *BRAF* mutations is possible (12-14). Although both *KRAS* and *BRAF* mutations activate the extracellular signal-regulated kinase (*ERK*) pathway and were thus hypothesized to provide redundant oncogenic stimuli, newer data suggest that this is not the case. Specifically, due to a paradoxical parallel activation of cellular senescence mechanisms, *KRAS* mutations have a much lower oncogenic capacity than *BRAF* mutations (15). For example, the presence of the *BRAF* V600E mutation results in a 138-fold increase in transforming (oncogenic) capability, which considerably exceeds the effects of *KRAS* G12V point mutations (16, 17). In turn, as the *KRAS* G12V mutation is reportedly the most prognostic genetic marker in CRLM, it follows that the *BRAF* V600E mutation will in combination exert an even more powerful effect on survival. Moreover, the coexistence of *KRAS* and *BRAF* mutations is thought to have a synergistic effect on disease progression (18, 19). Historically the recommendation for CRC has been to test for *BRAF* mutations only if the presence of *KRAS* mutation had already been excluded (9). As such, very limited information exists on the biological behavior of tumors that harbor concurrent *KRAS*/*BRAF* mutations. To the best of our knowledge, the present case series is the first to report on the implications of concurrent *KRAS*/*BRAF* mutations among surgically treated patients, and the largest set of surgical CRLM patients with data on the *KRAS*/*BRAF* mutational status thus far.

Case Series

The four cases presented in this report were derived from an international, multi-institutional cohort of 820 patients that underwent hepatic resection for CRLM at the Johns Hopkins Hospital, the Medical University of Vienna, the Stanford University School of Medicine, the Charite – University of Berlin, the Medical University of Graz, the Haukeland University Hospital (Bergen, Norway) and the Cleveland Clinic. Genomic DNA was isolated from primary CRC or CRLM tissue specimens and was used as a template for sequencing the *BRAF* gene locus (V600E and non-V600E mutations) and *KRAS* codons 12, 13 and 61, using standard techniques. Specifically, sequencing was performed using version 2 of Ion AmpliSeq multiplex panel (20).

Case 1. The first case was a 61-year-old Caucasian man with metachronous CRLM that presented after the resection of T2N0 primary CRC (left colon); the disease-free interval was 11 months. Prior to hepatic resection, the patient received three cycles of folinic acid, fluorouracil and oxaliplatin (FOLFOX), with the last treatment administered 6 weeks prior to surgery. Following minor hepatectomy, a major pathological response to chemotherapy was noted (defined as the presence of 0-49% viable tumor in the resected specimen). According to the pathology report, a single tumor of 0.7 cm was found in the specimen and an R0 resection was achieved. The serum carcinoembryonic antigen (CEA) level at the time of hepatic resection was 13.4 ng/ml. A G13D mutation of the *KRAS* gene was detected. A codon 600 mutation in exon 15 of the *BRAF* gene, resulting in the substitution of valine with glutamine (V600E) was also detected. In addition, the patient was positive for mutations of phosphatase and tensin homolog (*PTEN*), mothers against decapentaplegic homolog 4 (*SMAD4*), smoothened (*SMO*) and tumor protein p53 (*TP53*), but negative for microsatellite instability (MSI). Following resection of CRLM, the patient remained in hospital for 12 days, with recovery complicated by abdominal dehiscence (Dindo–Clavien Grade 3). No post-hepatectomy chemotherapy was given. The patient experienced a single intrahepatic recurrence 290 days post-resection that was treated with chemotherapy. Unfortunately, the patient died from his disease 485 days post-hepatic resection.

Case 2. The second case was a 58-year-old Caucasian man diagnosed with synchronous CRLM and T3N1 primary CRC (sigmoid colon, two metastatic lymph nodes). Prior to undergoing major hepatectomy, the patient was treated with two cycles of FOLFOX, with the last cycle administered 5 weeks prior to resection. According to the pathology report, a single tumor of 0.4 cm was found in the specimen and an R0 resection (margin width of 8 mm) was achieved. Serum CEA at the time of hepatic resection was 8.5 ng/ml. Genetic sequencing of the liver lesions revealed the presence of a G12V mutation of the *KRAS* gene. A V600E mutation of the *BRAF* gene was also detected, but the patient was MSI negative. After resection, the patient spent 10 days in hospital. Of note, he did not receive any adjuvant treatment. The patient unfortunately succumbed to his disease 236 days post-hepatic resection.

Case 3. The third case was a 70-year-old Caucasian man diagnosed with synchronous CRLM and T4N2 primary CRC (rectum, 18 metastatic lymph nodes). The liver metastases were treated with a major hepatectomy. According to the pathology report, multiple, bilateral liver metastases were present throughout the specimen with the largest one measuring 0.3 cm; microscopic invasion of the resection

margin was also detected. Serum CEA at the time of hepatic resection was 6255.4 ng/ml. A G13D mutation of the *KRAS* gene and a D594N mutation of the *BRAF* gene were detected. The patient was also positive for an E545K mutation of the phosphatidylinositol-4,5-*bis*phosphate 3-kinase catalytic subunit alpha (*PIK3CA*) gene but negative for MSI. The patient spent 6 days in hospital after resection and experienced no postoperative complications. The patient was subsequently diagnosed with peritoneal disease and died 79 days post-hepatic resection.

Case 4. The fourth case was a 65-year-old man diagnosed with metachronous CRLM and T4N1 primary CRC (cecum, one metastatic lymph node). The patient was treated with minor hepatectomy; a solitary liver metastasis measuring 2 cm was noted in the specimen during pathological examination and an R0 resection (4 mm margin width) was achieved. Serum CEA at the time of hepatic resection was 4.1 ng/ml. A G12S mutation of the *KRAS* gene was observed. A G469A mutation of the *BRAF* gene. The patient remained in the hospital for 3 days and experienced no post-operative complications. After resection, the patient was treated with FOLFOX. As of the most recent follow-up visit 416 days after hepatic resection, the patient remains alive and disease-free.

Discussion

In this case series, we sought to describe the presentation and outcome of four surgically treated patients with CRLM and concurrent *KRAS*/*BRAF* mutations. Importantly, the present study is, to our knowledge, the first to focus exclusively on patients with surgically treated disease, and the largest set of such patients described thus far.

The majority of relevant studies have reported a low incidence of concomitant *KRAS* and *BRAF* mutations in CRC. For example, eight prior studies identified only four patients with concurrent *KRAS*/*BRAF* mutations out of a total cohort of 6251 patients (0.064%) (13, 21-27). Interestingly, Oliveira *et al*. reported a higher incidence of concurrent *KRAS*/*BRAF* mutations among patients with microsatellite stable sporadic CRC (10/250, 4%). With respect to CRLM specifically, only four cases of concomitant *KRAS*/*BRAF* mutations have been previously reported, all of which concerned medically treated patients (12, 13). Conversely, the present study provides insight into the incidence of combined *KRAS*/*BRAF* mutations in a surgical cohort. This distinction is important as surgical cohorts generally include patients with less aggressive disease characteristics. As such, it may be expected that combined *KRAS*/*BRAF* mutations would be extremely rare in surgical cohorts, given their reported synergistic effect on disease progression. However, the incidence of concurrent *KRAS*/*BRAF* mutations in our cohort was less rare than expected (4/820, 0.5%), possibly suggesting a lack of uniformity in the biological behavior of these mutations. Specifically, while Oliveira *et al*. and others have suggested that concurrent *KRAS*/*BRAF* mutations are associated with advanced disease stage, higher likelihood of lymph node involvement and distant metastasis, and reduced prognosis, our findings suggest that this pattern may not be universal; for example, our first case presented with relatively low-stage disease (12, 14).

The genetic profile of case 1 is notable for multiple reasons. Firstly, all concomitant *KRAS* and *BRAF* mutations described so far have exclusively involved KRAS codon 12 (12-14, 19). However, cases 1 and 3 harbored mutations of *KRAS* codon 13. Indeed, it is known that *KRAS* mutations generally involve codons 12 and 13, while codons 61 and 146 are affected more rarely (28). As such, while this is, to our knowledge, the first time that a *KRAS* codon 13 mutation has been described alongside a *BRAF* mutation, the involvement of codon 13 is broadly consistent with previous reports on *KRAS* mutations (7, 10, 29). Secondly, while in the current study we describe tumors with concomitant *KRAS* and both V600E and non-V600E mutations of *BRAF*, previous reports in CRLM have only described concomitant *KRAS*/*BRAF* V600E mutations. Interestingly, all of these reports have associated the presence of the *BRAF* V600E mutation with advanced disease stage, while in the present study, case 1 which harbored a V600E mutation presented with relatively low-stage disease. In contrast, cases 3 and 4 presented with advanced disease (T4N1), despite harboring non-V600E mutations.

The present case series was derived from a large multi-institutional cohort with readily available clinical and pathological data. However, the study was retrospective in nature, which may limit the generalizability of our findings. Prospective studies are needed to assess the true frequency and impact of concomitant *KRAS*/*BRAF* mutations in patients with CRLM. More importantly, given that both codon (codon 12 *vs*. 13) and point-specific *KRAS* mutations, as well as codon-specific *BRAF* mutations (V600E *vs*. non-V600E), reportedly have a variable impact on survival, it would be interesting to assess how different mutation combinations may impact prognosis (7).

In conclusion, the present case series suggests that the incidence of concomitant *KRAS*/*BRAF* mutations in surgical cohorts may be higher than previously hypothesized. Moreover, while the small sample size precludes reliable prognostic comparisons, the patients assessed had variable survival outcomes, rather than a uniformly adverse prognosis as previously reported. In the absence of larger studies, there is insufficient evidence to suggest that a change in either genetic testing strategies (*i.e*. testing for *BRAF* mutations in patients with *KRAS*-mutated tumors) or in the surgical management of patients with concomitant *KRAS*/*BRAF* mutations would result in any clinical benefit.

References

1 Siegel RL, Miller KD, Fedewa SA, Ahnen DJ, Meester RGS, Barzi A and Jemal A: Colorectal cancer statistics, 2017. CA Cancer J Clin *67*: 177-193, 2017.

2 Haddad AJ, Bani Hani M, Pawlik TM and Cunningham SC: Colorectal liver metastases. Int J Surg Oncol *2011*: 285840, 2011.

3 Zakaria S, Donohue JH, Que FG, Farnell MB, Schleck CD, Ilstrup DM and Nagorney DM: Hepatic resection for colorectal metastases: value for risk scoring systems? Ann Surg *246*: 183-191, 2007.

4 Spolverato G, Ejaz A, Azad N and Pawlik TM: Surgery for colorectal liver metastases: The evolution of determining prognosis. World J Gastrointest Oncol *5*: 207-221, 2013.

5 Tosi F, Magni E, Amatu A, Mauri G, Bencardino K, Truini M, Veronese S, De Carlis L, Ferrari G, Nichelatti M, Sartore-Bianchi A and Siena S: Effect of *KRAS* and *BRAF* mutations on survival of metastatic colorectal cancer after liver resection: a systematic review and meta-analysis. Clinical colorectal cancer *16*: e153-e163, 2017.

6 Barras D: *BRAF* mutation in colorectal cancer: an update. Biomark Cancer *7*: 9-12, 2015.

7 Margonis GA, Kim Y, Spolverato G, Ejaz A, Gupta R, Cosgrove D, Anders R, Karagkounis G, Choti MA and Pawlik TM: Association between specific mutations in *KRAS* codon 12 and colorectal liver metastasis. JAMA Surg *150*: 722-729, 2015.

8 Margonis GA, Spolverato G, Kim Y, Karagkounis G, Choti MA and Pawlik TM: Effect of *KRAS* mutation on long-term outcomes of patients undergoing hepatic resection for colorectal liver metastases. Ann Surg Oncol *22*: 4158-4165, 2015.

9 Tan C and Du X: *KRAS* mutation testing in metastatic colorectal cancer. World J Gastroenterol *18*: 5171-5180, 2012.

10 Vauthey JN, Zimmitti G, Kopetz SE, Shindoh J, Chen SS, Andreou A, Curley SA, Aloia TA and Maru DM: *RAS* mutation status predicts survival and patterns of recurrence in patients undergoing hepatectomy for colorectal liver metastases. Ann Surg *258*: 619-626; discussion 626-617, 2013.

11 Rajagopalan H, Bardelli A, Lengauer C, Kinzler KW, Vogelstein B and Velculescu VE: Tumorigenesis: *RAF/RAS* oncogenes and mismatch-repair status. Nature *418*: 934, 2002.

12 Larki P, Gharib E, Yaghoob Taleghani M, Khorshidi F, Nazemalhosseini-Mojarad E and Asadzadeh Aghdaei H: Coexistence of *KRAS* and *BRAF* mutations in colorectal cancer: a case report supporting the concept of tumoral heterogeneity. Cell J *19*: 113-117, 2017.

13 Sahin IH, Kazmi SM, Yorio JT, Bhadkamkar NA, Kee BK and Garrett CR: Rare though not mutually exclusive: A report of three cases of concomitant kras and braf mutation and a review of the literature. J Cancer *4*: 320-322, 2013.

14 Vittal A, Middinti A and 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*: 2321052, 2017.

15 Long GV, Menzies AM, Nagrial AM, Haydu LE, Hamilton AL, Mann GJ, Hughes TM, Thompson JF, Scolyer RA and Kefford RF: Prognostic and clinicopathologic associations of oncogenic *BRAF* in metastatic melanoma. J Clin Oncol *29*: 1239-1246, 2011.

16 Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR and Futreal PA: Mutations of the *BRAF* gene in human cancer. Nature *417*: 949-954, 2002.

17 Oikonomou E, Makrodouli E, Evagelidou M, Joyce T, Probert L and Pintzas A: *BRAF*(V600E) efficient transformation and induction of microsatellite instability *versus KRAS*(G12V) induction of senescence markers in human colon cancer cells. Neoplasia *11*: 1116-1131, 2009.

18 Morkel M, Riemer P, Blaker H and Sers C: Similar but different: distinct roles for *KRAS* and *BRAF* oncogenes in colorectal cancer development and therapy resistance. Oncotarget *6*: 20785-20800, 2015.

19 Oliveira C, Velho S, Moutinho C, Ferreira A, Preto A, Domingo E, Capelinha AF, Duval A, Hamelin R, Machado JC, Schwartz S Jr., Carneiro F and Seruca R: *KRAS* and *BRAF* oncogenic mutations in MSS colorectal carcinoma progression. Oncogene *26*: 158-163, 2007.

20 Malapelle U, Vigliar E, Sgariglia R, Bellevicine C, Colarossi L, Vitale D, Pallante P and Troncone G: Ion torrent next-generation sequencing for routine identification of clinically relevant mutations in colorectal cancer patients. J Clin Pathol *68*: 64-68, 2015.

21 Bokemeyer C, Bondarenko I, Hartmann JT, de Braud F, Schuch G, Zubel A, Celik I, Schlichting M and Koralewski P: Efficacy according to biomarker status of cetuximab plus FOLFOX-4 as first-line treatment for metastatic colorectal cancer: the OPUS study. Ann Oncol *22*: 1535-1546, 2011.

22 De Roock W, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilas G, Kalogeras KT, Kotoula V, Papamichael D, Laurent-Puig P, Penault-Llorca F, Rougier P, Vincenzi B, Santini D, Tonini G, Cappuzzo F, Frattini M, Molinari F, Saletti P, De Dosso S, Martini M, Bardelli A, Siena S, Sartore-Bianchi A, Tabernero J, Macarulla T, Di Fiore F, Gangloff AO, Ciardiello F, Pfeiffer P, Qvortrup C, Hansen TP, Van Cutsem E, Piessevaux H, Lambrechts D, Delorenzi M and Tejpar S: 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 *11*: 753-762, 2010.

23 Lamy A, Blanchard F, Le Pessot F, Sesboue R, Di Fiore F, Bossut J, Fiant E, Frebourg T and Sabourin JC: Metastatic colorectal cancer *KRAS* genotyping in routine practice: results and pitfalls. Mod Pathol *24*: 1090-1100, 2011.

24 Li HT, Lu YY, An YX, Wang X and Zhao QC: *KRAS, BRAF* and *PIK3CA* mutations in human colorectal cancer: Relationship with metastatic colorectal cancer. Oncol Rep *25*: 1691-1697, 2011.

25 Maughan TS, Adams RA, Smith CG, Meade AM, Seymour MT, Wilson RH, Idziaszczyk S, Harris R, Fisher D, Kenny SL, Kay E, Mitchell JK, Madi A, Jasani B, James MD, Bridgewater J, Kennedy MJ, Claes B, Lambrechts D, Kaplan R, Cheadle JP and Investigators MCT: Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: Results of the randomised phase 3 MRC COIN trial. Lancet *377*: 2103-2114, 2011.

26 Price TJ, Hardingham JE, Lee CK, Weickhardt A, Townsend AR, Wrin JW, Chua A, Shivasami A, Cummins MM, Murone C and Tebbutt NC: Impact of *KRAS* and *BRAF* gene mutation status on outcomes from the phase III AGITG MAX trial of capecitabine alone or in combination with bevacizumab and mitomycin in advanced colorectal cancer. J Clin Oncol *29*: 2675-2682, 2011.

27 Van Cutsem E, Kohne CH, Lang I, Folprecht G, Nowacki MP, Cascinu S, Shchepotin I, Maurel J, Cunningham D, Tejpar S, Schlichting M, Zubel A, Celik I, Rougier P and Ciardiello F: 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 *29*: 2011-2019, 2011.

28 Seth R, Crook S, Ibrahem S, Fadhil W, Jackson D and Ilyas M: Concomitant mutations and splice variants in *KRAS* and *BRAF* demonstrate complex perturbation of the RAS/RAF signalling pathway in advanced colorectal cancer. Gut *58*: 1234-1241, 2009.

29 Margonis GA, Kim Y, Sasaki K, Samaha M, Amini N and Pawlik TM: Codon 13 *KRAS* mutation predicts patterns of recurrence in patients undergoing hepatectomy for colorectal liver metastases. Cancer *122*: 2698-2707, 2016.

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