Case Report

Coexistence of KRAS and BRAF Mutations in Colorectal Cancer: A Case Report Supporting The Concept of Tumoral Heterogeneity

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Abstract

The detection of KRAS and BRAF mutations is a crucial step for the correct therapeutic approach and predicting the epidermal growth factor receptor (EGFR)-targeted therapy resistance of colorectal carcinomas. The concomitant KRAS and BRAF mutations occur rarely in the colorectal cancers (CRCs) with the prevalence of less than 0.001% of the cases. In patients with KRAS-mutant tumors, BRAF mutations should not regularly be tested unless the patient is participating in a clinical trial enriching for the presence of KRAS or BRAF-mutated tumor. The current report demonstrates a case with advanced adenocarcinoma of the colon showing the coexistence of KRAS and BRAF mutations and may have profound clinical implications for disease progression and therapeutic responses.

Keywords: BRAF, CRC, EGFR, KRAS

Introduction

Colorectal cancer (CRC) is one of the most common cancers, especially in the developed countries and its worldwide mortality rates exceed 700,000 deaths per year. CRC usually causes by the mutations in the epithelial cells of the gastrointestinal surfaces resulting in hyperactivity of the signaling pathways and finally transforms these cells into the adenomatous polyps. Accumulation of the inherited or acquired mutations transits the adenomatous polyps to malignancy. The genetic mutations and morphological changes of this progression path, have been well described in the previous studies (1, 2). Significant genetic heterogeneity implicated in the CRC tumors, varying from the heterogeneous somatic DNA mutations to the chromosomal imbalances and even epigenetic factors such as DNA methylation (3). The current consensus theory of the heterogeneity of the CRC tumors has been underscored in most researches and highlighting the differences of the mutational status of KRAS gene between the primary and metastatic tumors (3-5).

KRAS protein is a key factor in the regulation of cell mitosis. KRAS activates its primary downstream target protein named BRAF, a serine-threonine protein kinase that mediates the KRAS signal toward the downstream effectors, Mitogen-activated protein (MAP) kinase extracellular signal-regulated kinase (MEK) and the extracellular signal-regulated kinase (ERK) (6). In
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normal situations, wild-type KRAS provokes cell cycle progression. However, it could act as a gate keeper throughout tumor growth and block cancer development (7). Mutations in the gene encoding KRAS, would change its ability in hydrolyzing GTP to GDP and results in constantly activated KRAS protein, as previously reported (8, 9). The impact of KRAS abnormal activity in cancer cells invasion has been well described by Liao et al. (10) as permanent activated KRAS, upregulates the metalloproteinase 2 (MMP2) expression level, and may promote cancer aggressiveness. Also, the elevated level of mutated KRAS enhances the carcinoembryonic antigen (CEA) level and blocks the monolayer formation of colon epithelial cells (11).

The mutations in KRAS and BRAF genes as the key mediators in the epidermal growth factor receptor (EGFR) signaling pathways play an important role in the colorectal pathogenesis and are associated with the primary resistance to the EGFR inhibitors (12-15). KRAS mutations as one of the most and best-described prognostic factors in the prediction of the resistance to EGFR-targeting therapeutic agents, have been reported in nearly 40% of the patients affected with the CRC (16-20). The vast majority of the KRAS mutations occur in the codons 12 and 13 of the exon 2, whereas the remainders occur mostly in the codons 61 and 146. BRAF mutations have been recently reported in 5 to 15% of CRC cases (21-23). Beside the KRAS mutations, it has recently been shown that there is a similar association between the BRAF mutations and resistance to EGFR-targeting agents in CRC patients (17, 24, 25). However, the likelihood of the presence of two or more mutations in the same codon of the KRAS gene is too rare and the reports of these co-occurrence mutations have little been documented in colorectal cancer. In addition, the clinical consequences and prognostic values of the multiple mutations in the CRC patients have not been fully elucidated yet (15, 26-28). Up to now, the joint mutant alterations in KRAS and BRAF sequences in a patient seem very rare (17). However, this study reports a case of the coexistence of the two somatic mutations in the codon 12 of the exon 2 of the KRAS gene and the codon V600 of the BRAF gene in a 50-year-old man affected with an advanced adenocarcinoma of the rectum. This supports the possibility of the colorectal tumors originated from two clonal origins with the coexistence of different mutations at the same genetic level.

Case report

A 50-year-old male patient suffering from the tenesmus and the presence of the blood in his stool, was admitted to the Gastroenterology and Liver Disease Research Institute, Taleghani Hospital, Tehran, Iran in January 2013. He referred with no personal or family history of the colorectal carcinoma or other related gastrointestinal diseases. Following the colonoscopy procedure, a hyperemic and flat lesion in the descending colon was discovered. The colon biopsy was performed to evaluate the depth of invasion in colon carcinoma in combination with the clinical examination by computed tomography scan. The final diagnosis was advanced colon cancer with liver metastases T3N2M1, stage IV.

After obtaining informed consent from patient, the EGFR immunohistochemical expression profile was investigated by anti-EGFR monoclonal antibody according to the manufacturer’s descriptions. The paraffin-embedded tissue sections were collected on microscopic slides. Tumor and tumor-free areas of the samples were identified within 15 μm thick deparaffinized, lightly hematoxylin-counterstained sections and subjected to the pathological studies. Each microdissected area was applied to the DNA extraction followed by the mutational analysis of KRAS (exons 2 and 3) and BRAF (exon 15) by using polymerase chain reaction (PCR) and direct sequencing. PCR cycling conditions were performed according to the manufacturer’s instruction. The ABI 3130 genetic analyzer (Applied Biosystems, USA) and the Big Dye Terminator (Applied Biosystems, USA) were used for the sequencing reactions of this study.

To investigate microsatellite instability (MSI), the DNA samples from the blood and microdissected tumoral areas of the patient were examined by using the BAT26, BAT25, NR24, NR21 and NR27 mononucleotide microsatellite markers. These markers are known to be the most sensitive markers of MSI status and widely uses for identification of the CRC cases with the concomitant mismatch repair (MMR) defects.
KRAS and BRAF mutational status analysis was performed on the representative lesion of the colon, using the PCR-sequencing method and MSI analysis. The identified mutations in KRAS and BRAF genes were a mutation in codon 12 of exon 2 and a missense nucleotide base change in codon 600 of exon 15 (GTG to GAG), respectively. However, MSI was not detected.

**Discussion**

CRC is estimated as the third commonly diagnosed type of cancer and the fourth common cause of the cancer mortality after the lung, stomach and liver cancers, in both males and females. Considering the invention of new therapeutics including Irinotecan and Oxaliplatin in addition to the development of targeted therapies such as Cetuximab and Bevacizumab, the survival rates of diagnosed patients have been significantly improved. It has been shown in several experiments that the KRAS mutations in primary colorectal tumors play a prognostic role as a predictor of resistance to the anti-EGFR antibodies (12, 18, 19). Although the results of the KRAS mutational analysis of the primary tumor usually match with the metastases in only 5 to 10% of the cases, the KRAS mutational status is heterogeneous between the primary tumors and the metastases (5, 29-32). This observation may reflect the increased genetic instability in cells that progressively acquiring mutations or the presence of a heterogeneous group of the neoplastic cells inside the tumor (33, 34). Moreover, the co-occurrence of KRAS and BRAF mutations in the same colorectal tumor has been reported in few studies. These studies have also reported the correlation of the concomitant KRAS and BRAF mutations with the clinical and morphological characteristics (15, 26-28).

Because of the rarity of this observation, it is not clear whether the concomitant KRAS and BRAF mutant tumors have a different biology and natural history than singly KRAS or BRAF mutant tumors, or which of the two mutations play the dominant role in driving the tumor proliferation. It has been noticed that the proportion of the concomitant mutations is associated with the degree of transmural invasion of the tumor; moreover, 2.8 and 9.4% of concomitant mutations occur in the T2 and T4 tumors, respectively, suggesting the activation role of both genes in the tumor progression (35). Molecular profiling studies proved that the KRAS and BRAF mutant tumors have different mutation results and probably depict the different over-activated signaling cascades (36). Currently, it is not obvious that which gene expression profile pattern is dominant (35). Considering the mutational characteristics of the CRC, including the high degree of tumor heterogeneity and the vast variety of the mutations along with the epigenetic alterations, it is likely that the gene expression profiling of these tumors will provide therapeutics benefits in terms of better understanding and determining activation of the different signaling pathways in tumors and applying the obtained prognostic biomarkers in the management and treatment of the disease. The current study supports the polyclonal and intratumoral heterogeneity features of the colon cancer, in which a mixture of cell clones with varying mutations characterizes the tumors. The coexistence of distinct clones with the high degree of the mutational heterogeneity could bring about profound clinical indications in terms of the disease progression, prognostic features and responses to particular therapeutic regimens. In particular, the present case appears to support the hypothesis that the co-occurrence of KRAS and BRAF mutations is associated with more aggressive clinical manifestations.

**Conclusion**

Currently, KRAS mutation status and microsatellite instability analysis are the only well-explained prognostic biomarkers as the negative predictors of the therapy efficiency in colorectal cancer. On the other hand, concomitant KRAS and BRAF-mutated colorectal tumors are relatively rare, so that the routine analysis of BRAF mutations in tumors with the KRAS mutations is not recommended. The case shows, that the concomitant KRAS and BRAF mutations is associated with more severe disease. This may provide clinical implications for cancer progression and management. Our case study suggests that the analysis of BRAF mutation, especially in KRAS-positive tumors would be highly advisable. In addition, the future clinical trials related to
colorectal cancer, should specifically consider the eligibility of patients with concomitant KRAS and BRAF-mutated tumors.

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References

1. Fearon ER, Dang CV. Cancer genetics: tumor suppressor meets oncogene. Curr Biol. 1999; 9(2): R62-65.
2. Chung DC. The genetic basis of colorectal cancer: insights into critical pathways of tumorigenesis. Gastroenterology. 2000; 119(3): 854-865.
3. Gerlinger M, Rowan AJ, Horswill S, Larkin J, Endesfelder D, Gronroos E, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med. 2012; 366(10): 883-892.
4. Marusyk A, Polyak K. Tumor heterogeneity: causes and consequences. Biochim Biophys Acta. 2010; 1805(1): 105-117.
5. Baldus SE, Schafer KL, Engers R, Hartlieb D, Stoecklein NH, Gabbert HE. Prevalence and heterogeneity of KRAS, BRAF, and PIK3CA mutations in primary colorectal adenocarcinomas and their corresponding metastases. Clin Cancer Res. 2010; 16(3): 790-799.
6. Dhomem N, Marais R. New insight into BRAF mutations in cancer. Curr Opin Genet Dev. 2007; 17(1): 31-39.
7. Zhang Z, Wang Y, Vikis HG, Johnson L, Liu G, Li J, et al. Wildtype Kras2 can inhibit lung carcinogenesis in mice. Nat Genet. 2001; 29(1): 25-33.
8. McCoy MS, Toole JJ, Cunningham JM, Chang EH, Lowy DR, Weinberg RA. Characterization of a human colon/lung carcinoma oncogene. Nature. 1983; 302(5903): 79-81.
9. Kranebogen O, The KRAS oncogene: past, present, and future. Biochem Biophys Acta. 2005; 1752(2): 81-82.
10. Liao J, Wolfman JC, Wolfman A. K-ras regulates the steady-state expression of matrix metalloproteinase 2 in fibroblasts. J Biol Chem. 2003; 278(34): 31871-31878.
11. Yan Z, Deng X, Chen M, Xu Y, Ahram M, Sloane BF, et al. Oncogenic c-Ki-ras but not oncogenic c-Ha-ras up-regulates CEA expression and disrupts basolateral polarity in colon epithelial cells. J Biol Chem. 1997; 272(44): 27902-27907.
12. Amado RG, Wolf M, Peeters M, Van Cutsem E, Siena S, Freeman DJ, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol. 2008; 26(10): 1626-1634.
13. Bokemeyer C, Bondarenko I, Makhsoum A, Hartmann JT, Aparicio J, de Braud F, et al. Fluorouracil, leucovorin, and irinotecan in combination with cetuximab in metastatic colorectal cancer. J Clin Oncol. 2008; 26(5): 663-671.
14. Bokemeyer C, Bondarenko I, Hartmann J, de Braud FG, Volovat C, Nippogen J, et al. KRAS status and efficacy of first-line treatment of patients with metastatic colorectal cancer (mCRC) with FOLFOX with or without cetuximab: The OPUS experience. J Clin Oncol. 2008; 26(15_suppl): 4000.
15. De Roock W, Piessevaux H, De Schutter J, Janssens M, De Hertogh G, Personeni N, et al. KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. Ann Oncol. 2008; 19(3): 508-515.
16. Di Fiore F, Blanchard F, Charbonnier F, Le Pessod F, Lamy A, Galais MP, et al. Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated with Cetuximab plus chemotherapy. Br J Cancer. 2007; 96(8): 1168-1169.
17. Di Nicolantonio F, Martini M, Molinari F, Sartore-Bianchi A, Arena S, Saletti P, et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. J Clin Oncol. 2008; 26(35): 5705-5712.
18. Karapetis CS, Khambata-Ford S, Jonker DJ, O’Callaghan CJ, Tu D, Tebbutt NC, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med. 2008; 359(17): 1757-1765.
19. Liévre A, Bachet JB, Le Corre D, Boige V, Landi B, Emile JF, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res. 2006; 66(8): 3992-3995.
20. Oliveira C, Velho S, Moutinho C, Ferreira A, Preno A, Domingo E, et al. KRAS and BRAF oncogenic mutations in MSS colorectal carcinoma progression. Oncogene. 2007; 26(1): 158-163.
21. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF gene in human cancer. Nature. 2002; 417(6892): 949-954.
22. Rajagopalan H, Bardelli A, Lengauer C, Kinzler KW, Vogelstein B, Velculescu VE. Tumorigenesis: RAF/RAS oncopgenes and mismatch-repair status. Nature. 2002; 418(6901): 934.
23. Yuen ST, Davies H, Chan TL, Ho JW, Bignell GR, Cox C, et al. Similarity of the phenotypic patterns associated with BRAF and KRAS mutations in colorectal neoplasia. Cancer Res. 2002; 62(22): 6451-6455.
24. Pratillas CA, Hanrahan AJ, Halilovic E, Persaud Y, Soh J, Chitale D, et al. Genetic predictors of MEK dependence in non-small cell lung cancer. Cancer Res. 2008; 68(22): 9375-9383.
25. Solit DB, Garraway LA, Pratillas CA, Sawai A, Getz G, Basso A, et al. BRAF mutation predicts sensitivity to MEK inhibition. Nature. 2006; 439(7074): 358-362.
26. Jönsson M, Ekstrand A, Edelking T, Eberhard J, Grabau D, Borg D, et al. Experiences from treatment-predictive KRAS testing; high mutation frequency in rectal cancers from females and concurrent mutations in the same tumor. BMC Clin Pathol. 2009; 9: 8.
27. Moerkert K, Arends JW, van Driel M, de Bruijne A, ten Kate J. Type and number of Ki-ras point mutations relate to stage of human colorectal cancer. Cancer Res. 1994; 54(13): 3376-3378.
28. Sameer AS, Rehman SU, Pandith AA, Syyed N, Shah ZA, Chowdhri NA, et al. Molecular gatekeepers succumb to gene aberrations in colorectal cancer in Kashmiri population: an analysis of Ki-ras and p53 mutations. J Med. 1998; 119(3): 854-865.
29. Al-Mulla F, Going JJ, Bowden ET, Winter A, Pickford IR, Binme GD. Heterogeneity of mutant versus wild-type Ki-ras in primary and metastatic colorectal carcinomas, and association of codon-12 valine with early mortality. J Pathol. 1998; 185(2): 130-138.
30. Albanese I, Scibetta AG, Migliavacca M, Russo A, Bazan V, Tomasino RM, et al. Heterogeneity within and between primary colorectal carcinomas and matched metastases as revealed by analysis of Ki-ras and p53 mutations. Biochem Biophys Res Commun. 2004; 325(3): 784-791.
31. Mancuso A, Sollami R, Racine F, Cerbone L, Macciomei MC, Leone A. Patient with colorectal cancer with heterogeneous KRAS molecular status responding to cetuximab-based chemotherapy. J Clin Oncol. 2010; 28(36): e756-e758.

32. Lamy A, Blanchard F, Le Pessot F, Sesboüé R, Di Fiore F, Bossut J, et al. Metastatic colorectal cancer KRAS genotyping in routine practice: results and pitfalls. Mod Pathol. 2011; 24(8): 1090-1100.

33. Bouchahda M, Karaboué A, Saffroy R, Innominato P, Gorden L, Guettier C, et al. Acquired KRAS mutations during progression of colorectal cancer metastases: possible implications for therapy and prognosis. Cancer Chemother Pharmacol. 2010; 66(3): 605-609.

34. Andreyev HJ, Norman AR, Cunningham D, Oates JR, Clarke PA. Kirsten ras mutations in patients with colorectal cancer: the multicenter "RASCAL" study. J Natl Cancer Inst. 1998; 90(9): 675-684.

35. Sahin IH, Kazmi SM, Yorio JT, Bhadkamkar NA, Kee BK, 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. 2013; 4(4): 320-322.

36. Morkel M, Riemer P, Bläker H, Sers C. Similar but different: distinct roles for KRAS and BRAF oncogenes in colorectal cancer development and therapy resistance. Oncotarget. 2015; 6(25): 20785-20800.