BRAF Mutation in Colorectal Cancer: An Update

Supplementary Issue: Biomarkers for Colon Cancer

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ABSTRACT: Colorectal cancer (CRC) is still one of the deadliest cancer-related diseases. About 10% of CRC patients are characterized by a mutation in the B-Raf proto-oncogene serine/threonine kinase (*BRAF*) gene resulting in a valine-to-glutamate change at the residue 600 (V600E). This mutation is also present in more than 60% of melanoma patients. BRAF inhibitors were developed and found to improve patient survival; however, most patients at the end of the track ultimately develop resistance to these inhibitors. Melanoma patients benefit from the combination of BRAF inhibitors with mitogen/extracellular signal-regulated kinase (MEK) inhibitors, among others. Unfortunately, colorectal patients do not respond much efficiently, which suggests different resistance mechanisms between the two cancer types. This review aims at shedding light on recent discoveries that improve our understanding of the *BRAF* mutation biology in CRC.

KEYWORDS: BRAF, CRC, colorectal cancer, biomarker, oncogene

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Introduction

Colorectal cancer (CRC) remains the third leading cause of cancer-related deaths in the United States.[1] The initial theory stated by Vogelstein et al that relates the sequential multistep mutational process of CRC development is as important today as ever.[2] The proposed model describes the development of colorectal tumor through sequential activation of oncogenes and concomitant inactivation of tumor suppressors.[2] This model was refined recently by the so-called *Big Bang model*, stating that after an initial oncogenic mutation, within-tumor heterogeneity is favored by the development of subpopulations bearing other oncogenic mutational profiles.[3] Mutations in the *BRAF* gene are examples of such oncogenic events and are found in about 10% of CRC patients.[4] These mutations are associated with shorter progression-free and overall survival.[5,6] BRAF inhibitors have proven high efficiency in melanoma, in which *BRAF* mutation rate is more than 60%, with the response rates of 50%–80% in these patients.[7,8] High hopes were, therefore, raised on BRAF inhibitors for CRC *BRAF*-mutant treatment; however, such inhibitors revealed to be particularly ineffective with a response rate of approximately 5%.[9] One of the major topical clinical challenge in therapy against CRC remains to thwart resistance to BRAF inhibitors.[10] Since its discovery in 1988,[11] BRAF has been more widely studied with more than 6900 related publications listed in PubMed. Nevertheless, the fact remains that the lack of knowledge about BRAF biology still precludes CRC *BRAF*-mutant patients from being efficiently cured. This review aims at summarizing the current state of knowledge about the *BRAF* mutation in CRC, with an emphasis on the very recent advances made on BRAF inhibitors in therapies as well as their resistance.

BRAF and Mutated BRAF

The serine/threonine protein kinase BRAF is an important player in the epidermal growth factor receptor (EGFR)-mediated mitogen-activated protein kinase (MAPK) pathway, where it is activated by the RAS small GTPase.[12] The strength of BRAF, and also its extension to other RAF isoforms (ARAF and CRAF), is to not only activate the MAPK pathway that profoundly affects cell growth, proliferation, and differentiation but also affect other key cellular processes, such as cell migration (through RHO small GTPases), apoptosis (through the regulation of BCL-2), and survival (through the HIPPO pathway).[13] Thus, it is not a surprise that BRAF is found constitutively activated by mutation in 15% of all human known cancer types.[14] *BRAF* was reported to be mutated at several sites; however, the vast majority of mutated BRAF are V600E (1799T>A nucleotide change), characterizing up to 80% of all *BRAF* mutations.[14] This mutation results in amino acid change that confers constitutive kinase activity.[14] Most of the BRAF mutations result either in the acquirement of new phosphomimetic residues or in the release of the auto-inhibitory conformation imposed by the N-terminal region, which enhances the dimerization of the kinase domain, a crucial process for kinase activity. BRAF inhibitors have been

developed by different companies; the most commonly used are vemurafenib (marketed as Zelboraf by Roche) and dabrafenib (marketed as Tafinlar by GSK), but others exist such as LGX818 (encorafenib; Novartis), XL281 (Exelixis), and CEP-32496 (Ambit Biosciences Corporation).[15]

Colonic polyps can be classified into adenomatous polyps (~10%) and hyperplastic polyps (~90%). Hyperplastic polyps do not progress into CRC. Some polyps are called serrated polyps (WHO classification: ICD-O 8213/0) because of their saw-toothed morphology. These polyps were long considered as non-malignant, but this notion was challenged later. Serrated polyps are in turn subclassified into different types: serrated hyperplastic, traditional serrated adenomas (TSAs), or sessile serrated adenomas (SSAs).[16] TSA and SSA are considered premalignant. The transformation of epithelia into TSA and SSA polyps has been attributed to the *BRAF* mutation, thus defining this mutation as an early event in progression of CRC. The activation of WNT pathway in parallel with the inactivation of p53 and p16 only appear in the late development of CRC.[17] BRAF-mutated tumors are often right sided, more recurrent in woman, of higher grade, and associated with microsatellite instability (MSI) and old age.[18,19] MSI is a form of genetic instability because of the deficiency of the mismatch repair machinery and results in hypermutability. MSI has been attributed to be the most prognostic factor in CRC with instability conferring a better prognosis. Interestingly, the deleterious effects conferred by the *BRAF* mutation were found to be more pronounced in microsatellite stable patients compared to instable ones (MSI), although not statistically different.[18] The interaction between the *BRAF* status and the MSI status is a subject of intense debate. Proximal right-sided CRC is associated with a poorer prognosis.[20] The *BRAF* mutation is found highly enriched in right-sided proximal tumors.[21] The reason for this association is still incompletely understood.[22] For more detailed descriptions on molecular mechanisms and early discoveries concerning BRAF, the reader is redirected to a recent full-depth review.[12]

Predictive and Prognostic Role of Mutated BRAF

While the predictive role of KRAS mutation to cetuximab (an EGFR-blocking antibody) is well established, the predictive role of mutated *BRAF* is a subject of intense debate. Several studies have compared that the effect of anti-EGFR was beneficial in *BRAF* mutants. Nevertheless, until recently, none formally studied the effect of the acquisition of the *BRAF* mutation on the anti-EGFR response (ie, by comparing to *BRAF* wild-type patients). Recently, such a study was achieved by doing a meta-analysis that grouped eight cohorts consisting of 351 *BRAF*-mutant patients, including *BRAF* wild-type patients.[23] This analysis revealed that the hazard ratios of patients treated with EGFR-blocking antibodies (cetuximab or panitumumab) were not depending on the *BRAF* mutation status for overall survival (interaction test *P*-value: 0.43) but were close to significance for progression-free survival (interaction test *P*-value: 0.07).[23] The authors concluded that the *BRAF* mutation was not predictive of benefits provided by anti-EGFR therapies. Similarly, another meta-analysis reported by Pietrantonio et al revealed that EGFR-blocking antibodies did not increase the efficacy of standard chemotherapy in *BRAF*-mutant patients.[24] However, this study did not assess the survival differences between *BRAF* wild-type and *BRAF*-mutant patients.

On the contrary, the prognostic role of the *BRAF* mutation in CRC is well established and is usually associated with significant poorer prognosis.[5,6] For example, in a study involving more than 1200 stage II and III patients, a multivariate Cox proportional hazard regression indicated that the *BRAF* mutation significantly affects the overall survival (hazard ratio: 1.78 [1.15–2.76]; *P*-value: 0.01).[6] On the other hand, relapse-free survival was not found to be altered (hazard ratio: 1.30 [0.87–1.95]; *P*-value: 0.21).[6]

Resistance Against BRAF Inhibitors

The fact that the *BRAF* mutation is a bad predictor against anti-BRAF therapies suggests that the biology underlying this mutation is signaling through the MAPK pathway in a complex manner. Resistance to BRAF inhibitors is well known and is a subject of intense investigation. The vast majority of discoveries regarding resistance to BRAF inhibitors were achieved in melanoma because of the increased rate of BRAF V600E mutations. Currently, the field of CRC is updated with compelling discoveries.[25]

In melanoma, a plethora of resistance mechanisms to BRAF inhibitors were already discovered, including *MEK1*-, *MEK2*-, and *NRAS*-activating mutations; *BRAF* amplification; COT overexpression; platelet-derived growth factor receptor and EGFR overexpression; secondary *RAF*-related mutations; and the expression of constitutively active splicing variants of BRAF.[25,26] The vast majority of these escape mechanisms still tend to be related to the overactivation of the MAPK pathway, which is why the combination of anti-BRAF and anti-MEK is an intense subject of investigation. Recently, the Food and Drug Administration (FDA) approved the use of dabrafenib (a BRAF inhibitor) and trametinib (a MEK inhibitor) for BRAF-mutated unresectable or metastatic melanomas.[27]

Unfortunately, discoveries made on resistance to BRAF inhibitors in melanoma may not be easily applied in CRC because of the fact that anti-BRAF drug response rates are highly different in these cancers. Also, the difference in *BRAF* mutation incidence in these two types of cancer may indicate that BRAF signaling is not similarly required in these two cancer types and might be context dependent. In CRC, resistance to BRAF inhibitors was shown to be driven by feedback reactivation of EGFR that activates in turn MAPK via CRAF and RAS.[28,29] This activating feedback mechanism seems to be specific to CRC, as the majority of melanomas do not or only slightly express EGFR. These studies show that the feedback through EGFR could be efficiently prevented by

the combination of BRAF inhibitors, such as vemurafenib and dabrafenib with EGFR and MEK inhibitors, respectively. The combination of the BRAF inhibitor with the MEK inhibitor[28] or with the EGFR inhibitor[29] dramatically decreases mice tumor growth or even induces tumor reduction, while the single-agent effect was only minimal. These combinations are nevertheless known to ultimately lead to resistance.[30] The hepatocyte growth factor–mesenchymal–epithelial transition (MET) pathway is known to be involved in acquiring resistance to EGFR inhibitors through MET stimulation.[31] Interestingly, MET inhibition can bypass this resistance.[31]

A very recent study unraveled unanticipated resistance mechanisms by performing exon sequencing on tumors resistant to the combination of BRAF, MEK, and EGFR inhibitors.[30] This method revealed that resistant tumors exhibit *KRAS* and *BRAF* amplification as well as *MEK1* mutations resulting in MAPK activation, despite the treatment with these inhibitors.[30,32] Cells that became resistant to the drug cocktail maintained the *BRAF* mutation but displayed *KRAS* mutations as well (either G12D or G13D). This indicates that both mutations confer a selective advantage to the combination of BRAF, EGFR, and MEK inhibitors. However, it is well accepted that the appearance of simultaneous *BRAF* and *KRAS* mutations is an extremely infrequent event in natural colorectal tumors. The reason why it was unraveled very recently is because harboring both mutations confers negative selection for *BRAF*/*KRAS*-mutated cells that are more prone to senescence.[33] In 2013, Darrin Stuart and colleagues showed that *BRAF*-mutant melanoma become progressively addicted to vemurafenib and that drug cessation induced tumor shrinkage.[34] In this context, it could thus be appropriate to prescribe *drug holidays* after anti-BRAF therapy to treat *BRAF*-mutant patients.

Another mechanism that could explain the differential response of CRC to BRAF inhibitors versus melanoma is the levels of PI3K activation. In colorectal settings, PI3K was shown to be more consistently activated in cell lines compared to that in the melanoma settings.[35] Interestingly, it was found that anti-BRAF resistance could be bypassed by PI3K inhibition. Similarly, another study showed the generation of a genetically engineered mouse model for BRAF V600E colorectal and its subsequent treatment with combinatorial anti-BRAF and dual anti-PI3K/mTOR.[36] Tumor growth was found to be synergistically affected by the combination of both drugs. Altogether, the material reviewed so far suggests that the combinatorial approaches of multiple protein inhibitors in cancer therapy could be a potential strategy that raises high hopes for a better efficacy in future cancer treatments.

Future Aims and Discussion

The question of how to cure CRC *BRAF*-mutant patients remains unanswered at the moment but is currently in a full-scale investigation. A drug screening that was performed by Rad et al in 2013 in order to test the effects of drug combination on BRAF-mutated CRC cell lines confirmed that the combination of anti-BRAF with anti-MEK1/2 was an efficient strategy and also revealed that CHK1/2 and AURKA inhibitors, which target the cell cycle, and inhibitors of AKT, mTORC1/2, and PI3K were promising when combined with anti-BRAF.[17] One major goal would be to determine why all *BRAF*-mutant CRC cell lines do not respond equally to the different drug cocktails. CRC *BRAF*-mutant patients have been considered for a long time as one subtype with high clinical similarities. However, the plethora of anti-BRAF resistance mechanisms suggest that tumors can have different overall gene expression profiles. An effort to further classify the *BRAF*-mutant population based on gene expression is necessary to improve personalized therapy.

Several clinical trials are currently ongoing to elucidate which treatment has the best outcome. The most awaited results come from two different ongoing clinical trials that are assessing the combination of BRAF inhibitors with MEK and EGFR inhibitors (NCT01750918 in clinicaltrials.gov) or with PI3K inhibitor (NCT01719380 in clinicaltrials.gov). A case report assessing the anti-BRAF/EGFR combination already revealed exciting results with apparent clinical effects.[37] Another previous pilot study performed on 15 *BRAF*-mutant metastatic CRC patients with the aim to test the combination of BRAF and EGFR inhibitors revealed two things: first that this regimen was well tolerated and second that it resulted in modest efficiency, as tumor regression was observed in 10 of 12 evaluable patients.[38] One should, however, keep in mind that combinatorial approaches might result in unanticipated side effects.

Altogether, recent advances in the understanding of BRAF-related biology are encouraging, and it is indisputable that we have never been closer to elucidating the resistance mechanisms of mutated BRAF to currently used anticancer drugs. This should soon result in translational facts.

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REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin*. 2011;61(2):69–90.
2. Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal-tumor development. *N Engl J Med*. 1988;319(9):525–532.
3. Sottoriva A, Kang H, Ma Z, et al. A big bang model of human colorectal tumor growth. *Nat Genet*. 2015;47(3):209–216.
4. Tejpar S, Bertagnolli M, Bosman F, et al. Prognostic and predictive biomarkers in resected colon cancer: current status and future perspectives for integrating genomics into biomarker discovery. *Oncologist*. 2010;15(4):390–404.

5. Yuan ZX, Wang XY, Qin QY, et al. The prognostic role of BRAF mutation in metastatic colorectal cancer receiving anti-EGFR monoclonal antibodies: a meta-analysis. *PLoS One*. 2013;8(6):e65995.
6. Roth AD, Tejpar S, Delorenzi M, et al. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. *J Clin Oncol*. 2010;28(3): 466–474.
7. Flaherty KT, Puzanov I, Kim KB, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. *N Engl J Med*. 2010;363(9):809–819.
8. Long GV, Stroyakovskiy D, Gogas H, et al. Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. *N Engl J Med*. 2014;371(20): 1877–1888.
9. Kopetz S, Desai J, Chan E, et al. PLX4032 in metastatic colorectal cancer patients with mutant BRAF tumors [abstract]. *J Clin Oncol*. 2010;28(15s):abstr3534.
10. Cleary JM. *BRAF-Mutated Colorectal Cancer: Early Frustrations and Future Optimism*. 2014. Available at: http://www.onclive.com/publications/contemporary-oncology/2014/August-2014/BRAF-Mutated-Colorectal-Cancer-Early-Frustrations-and-Future-Optimism
11. Ikawa S, Fukui M, Ueyama Y, Tamaoki N, Yamamoto T, Toyoshima K. B-raf, a new member of the raf family, is activated by DNA rearrangement. *Mol Cell Biol*. 1988;8(6):2651–2654.
12. Lavoie H, Therrien M. Regulation of RAF protein kinases in ERK signalling. *Nat Rev Mol Cell Biol*. 2015;16(5):281–298.
13. Matallanas D, Birtwistle M, Romano D, et al. Raf family kinases: old dogs have learned new tricks. *Genes Cancer*. 2011;2(3):232–260.
14. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. *Nature*. 2002;417(6892):949–954.
15. Zhang W. BRAF inhibitors: the current and the future. *Curr Opin Pharmacol*. 2015;23:68–73.
16. Noffsinger AE. Serrated polyps and colorectal cancer: new pathway to malignancy. *Annu Rev Pathol*. 2009;4:343–364.
17. Rad R, Cadiñanos J, Rad L, et al. A genetic progression model of Braf(V600E)-induced intestinal tumorigenesis reveals targets for therapeutic intervention. *Cancer Cell*. 2013;24(1):15–29.
18. Lochhead P, Kuchiba A, Imamura Y, et al. Microsatellite instability and BRAF mutation testing in colorectal cancer prognostication. *J Natl Cancer Inst*. 2013; 105(15):1151–1156.
19. Gonsalves WI, Mahoney MR, Sargent DJ, et al; Alliance for Clinical Trials in Oncology. Patient and tumor characteristics and BRAF and KRAS mutations in colon cancer, NCCTG/Alliance N0147. *J Natl Cancer Inst*. 2014;106(7):dju106.
20. Meguid RA, Slidell MB, Wolfgang CL, Chang DC, Ahuja N. Is there a difference in survival between right- versus left-sided colon cancers? *Ann Surg Oncol*. 2008;15(9):2388–2394.
21. Missiaglia E, Jacobs B, D'Ario G, et al. Distal and proximal colon cancers differ in terms of molecular, pathological, and clinical features. *Ann Oncol*. 2014;25(10): 1995–2001.
22. Lee GH, Malietzis G, Askari A, Bernardo D, Al-Hassi HO, Clark SK. Is right-sided colon cancer different to left-sided colorectal cancer?—a systematic review. *Eur J Surg Oncol*. 2015;41(3):300–308.
23. Rowland A, Dias MM, Wiese MD, et al. Meta-analysis of BRAF mutation as a predictive biomarker of benefit from anti-EGFR monoclonal antibody therapy for RAS wild-type metastatic colorectal cancer. *Br J Cancer*. 2015;112(12): 1888–1894.
24. Pietrantonio F, Petrelli F, Coinu A, et al. Predictive role of BRAF mutations in patients with advanced colorectal cancer receiving cetuximab and panitumumab: a meta-analysis. *Eur J Cancer*. 2015;51(5):587–594.
25. Shi H, Hugo W, Kong X, et al. Acquired resistance and clonal evolution in melanoma during BRAF inhibitor therapy. *Cancer Discov*. 2014;4(1):80–93.
26. Charles J, Martel C, de FF, Leccia MT, Robert C, Busser B. [Mechanisms of resistance to anti-BRAF treatments]. *Ann Dermatol Venereol*. 2014;141(11):671–681.
27. Robert C, Karaszewska B, Schachter J, et al. Improved overall survival in melanoma with combined dabrafenib and trametinib. *N Engl J Med*. 2015;372(1): 30–39.
28. Prahallad A, Sun C, Huang S, et al. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature*. 2012; 483(7387):100–103.
29. Corcoran RB, Ebi H, Turke AB, et al. EGFR-mediated re-activation of MAPK signaling contributes to insensitivity of BRAF mutant colorectal cancers to RAF inhibition with vemurafenib. *Cancer Discov*. 2012;2(3):227–235.
30. Ahronian LG, Sennott EM, Van Allen EM, et al. Clinical acquired resistance to RAF inhibitor combinations in BRAF-mutant colorectal cancer through MAPK pathway alterations. *Cancer Discov*. 2015;5(4):358–367.
31. Della Corte CM, Fasano M, Papaccio F, Ciardiello F, Morgillo F. Role of HGF–MET signaling in primary and acquired resistance to targeted therapies in cancer. *Biomedicines*. 2014;2(4):345–358.
32. Corcoran RB, Dias-Santagata D, Bergethon K, Iafrate AJ, Settleman J, Engelman JA. BRAF gene amplification can promote acquired resistance to MEK inhibitors in cancer cells harboring the BRAF V600E mutation. *Sci Signal*. 2010; 3(149):ra84.
33. Cisowski J, Sayin VI, Liu M, Karlsson C, Bergo MO. Oncogene-induced senescence underlies the mutual exclusive nature of oncogenic KRAS and BRAF. *Oncogene*. 2015. doi:10.1038/onc.2015.186.
34. Das Thakur M, Salangsang F, Landman AS, et al. Modelling vemurafenib resistance in melanoma reveals a strategy to forestall drug resistance. *Nature*. 2013; 494(7436):251–255.
35. Mao M, Tian F, Mariadason JM, et al. Resistance to BRAF inhibition in BRAF-mutant colon cancer can be overcome with PI3K inhibition or demethylating agents. *Clin Cancer Res*. 2013;19(3):657–667.
36. Coffee EM, Faber AC, Roper J, et al. Concomitant BRAF and PI3K/mTOR blockade is required for effective treatment of BRAF(V600E) colorectal cancer. *Clin Cancer Res*. 2013;19(10):2688–2698.
37. Connolly K, Brungs D, Szeto E, Epstein RJ. Anticancer activity of combination targeted therapy using cetuximab plus vemurafenib for refractory BRAF (V600E)-mutant metastatic colorectal carcinoma. *Curr Oncol*. 2014;21(1): e151–e154.
38. Yaeger R, Cercek A, O'Reilly EM, et al. Pilot trial of combined BRAF and EGFR inhibition in BRAF-mutant metastatic colorectal cancer patients. *Clin Cancer Res*. 2015;21(6):1313–1320.