BRAF mutant colorectal cancer as a distinct subset of colorectal cancer: clinical characteristics, clinical behavior, and response to targeted therapies

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Abstract: Despite new and more effective cytotoxic chemotherapy, limitations to conventional agents have been reached in a subset of patients with advanced colorectal cancer (CRC). The identification of novel prognostic and predictive biomarkers to guide individualized treatment plans is critical to overcoming therapeutic resistance. Mutation of the BRAF proto-oncogene is linked to a variety of cancers and is increasingly being used as a prognostic tool and therapeutic target. This paper is a comprehensive review of the literature that summarizes the clinical, pathologic, and molecular features of BRAF mutated CRC that support the hypothesis that BRAF mutant cancers represent a distinct subset of CRC with its own clinical implications with regard to prognosis, treatments and emerging therapeutic strategies.

Keywords: Colorectal cancer (CRC); BRAF; prognostic biomarker; targeted therapy

Introduction

Despite improvements in screening and early detection, colorectal cancer (CRC) remains a leading cause of cancer death in the United States. Advances in molecular biology have increased our knowledge of the genetic and epigenetic events involved in tumorigenesis and have led to the development of novel targeted therapeutics. The Ras-Raf-mitogen-activated protein kinase (MAPK) signaling pathway has been implicated as a critical mediator of colorectal carcinogenesis. KRAS and NRAS mutations are present in 50% of CRCs and their therapeutic significance is well defined (1-3). Studies support the hypothesis that KRAS/NRAS mutations result in constitutive activation of the Ras-Raf-MAPK pathway, downstream of epidermal growth factor receptor (EGFR), rendering these tumors resistant to anti-EGFR therapies (4-7). These findings prompted the incorporation of KRAS/NRAS mutation status into the clinical treatment algorithm for CRC, and established the Ras-Raf-MAPK pathway as a principal target for the development of novel molecular therapeutic agents for the treatment of CRC (8,9).

BRAF, another potent modulator of the MAPK pathway, has recently emerged as a prognostic biomarker and promising new target for the treatment of CRC. Oncogenic mutations in BRAF are present in 10% of CRC. Studies demonstrate that carriers of BRAF mutations possess discrete clinical characteristics and oncologic outcomes (10-12). Furthermore, BRAF status is believed to be responsible for the 12-15% of patients who fail anti-EGFR (10,13,14). Because of its increasing significance, the National Comprehensive Cancer Network guidelines now recommend BRAF mutation testing in patients with
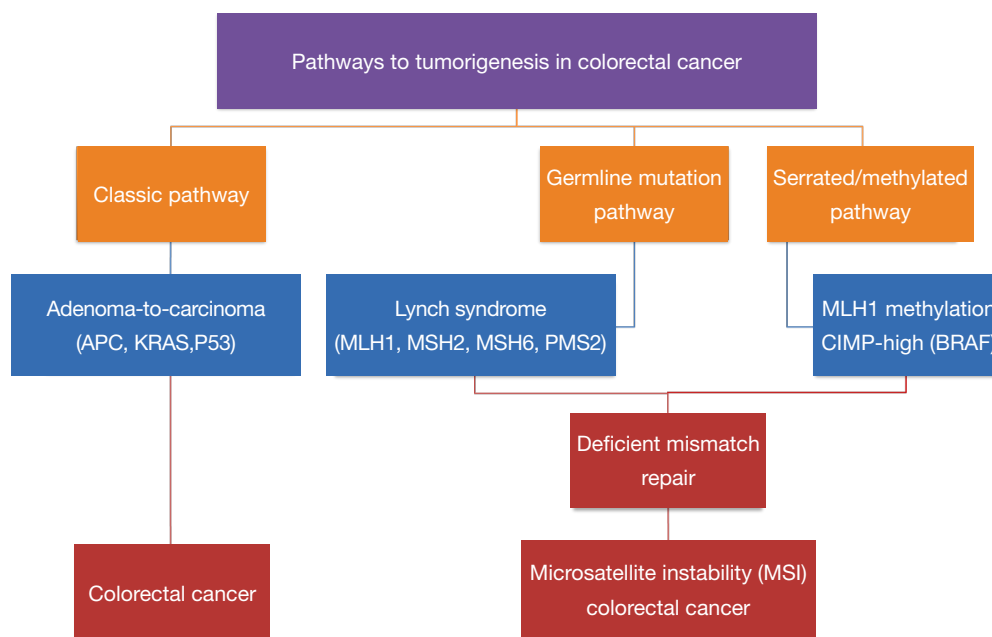
Figure 1 Molecular pathways leading to CRC. The classic pathway accounts for 30-70% of CRCs and results in the progression from adenoma to carcinoma. Germline mismatch repair mutations lead to Lynch Syndrome, which accounts for approximately one quarter of all MSI CRCs. These inherited mutations lead to carcinoma when a second hit occurs to result in loss of the wild-type copy. The Serrated/ Methylated pathway occurs due to the epigenetic inactivation of MLH1 by hypermethylation resulting in a sporadic MSI phenotype. CRC, colorectal cancer; MSI, microsatellite instability.

metastatic disease. In this article, we will review the role of BRAF mutations in the development of CRC and summarize the molecular and clinicopathologic features unique to this genetic subtype.

BRAF carcinogenesis pathway

It is widely accepted that there are multiple pathways that lead to the development of CRC (*Figure 1*). The classic adenoma-to-carcinoma pathway is typically seen with the loss of APC and/or p53 tumor suppressor genes with chromosomal instability (1). A second pathway involves the loss of DNA mismatch repair and is exemplified by the germline mutations seen in Lynch Syndrome (15,16). BRAF appears to act via a third pathway; the serrated/methylator pathway (17-19). These tumors are characterized by the methylation of CpG islands that cause the silencing of critical tumor suppressor genes and are termed CpG Island Methylator Phenotype (CIMP) tumors.
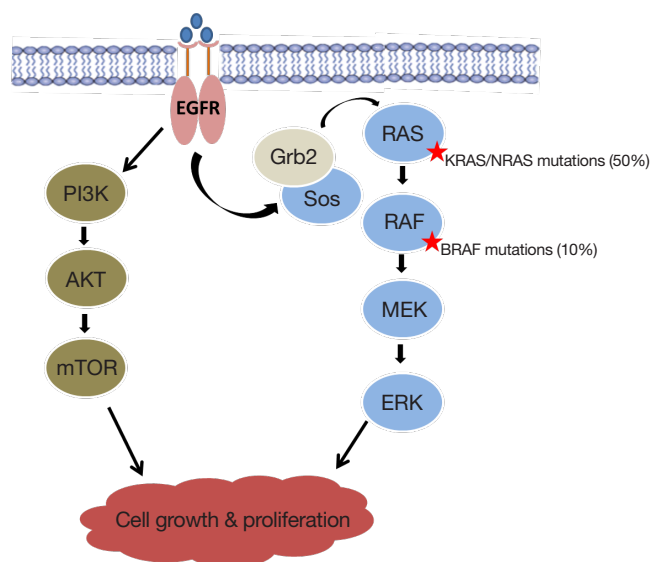
The BRAF oncogene codes for a serine/threonine kinase which acts downstream of KRAS in the MAPK pathway (*Figure 2*). BRAF mediates its effect by activating mitogen-activated protein kinase kinase (MAPKK or MEK), thus promoting cell proliferation. $BRAF^{V600E}$ is an activating mutation that accounts for approximately 90% of all BRAF mutations seen in CRC (3,20). It results from the transversion of thymidine to adenine at nucleotide 1799 in the kinase domain, causing a valine to glutamate substitution that leads to constitutive activation of MEK and uninhibited EGFR-independent cellular proliferation (10,21). BRAF and KRAS/NRAS mutations are mutually exclusive in CRC (3,13,14). This fact supports the hypothesis that BRAF is the principal effector of KRAS/ NRAS in the MAPK pathway and that both mutations have equivalent effects on tumorigenesis.

Clinicopathologic characteristics

Knowledge about the clinical implications of BRAF mutations in CRC is rapidly increasing, but it is already evident that BRAF mutated tumors comprise a discrete disease subtype with a unique patient population and associated prognosis. As previously discussed, BRAF mutant cancers are highly methylated (CIMP-high) when compared to BRAF wild-type tumors. Additionally, BRAF mutation is strongly associated with microsatellite instability (MSI).

Table 1 summarizes the incidence of MSI-high, CIMP-high and BRAF mutated tumors observed in a variety of CRC cohorts. In sporadic CRCs, BRAF mutation is seen in approximately 60% of MSI high tumors and only 5-10% of microsatellite stable (MSS) tumors (3,10,13,14). This is because BRAF^{V600E} mutation results in hypermethylation of the MLH1 gene promoter, resulting in loss of the tumor suppressor function and leading to diminished DNA mismatch repair (29-32). This occurs exclusive of the germline mismatch repair mutations seen in Lynch

Syndrome (*Figure 3*).

Phenotypically, BRAF mutated CRCs display different characteristics when compared to BRAF wild-type. Studies have demonstrated that BRAF mutant tumors are more prevalent in women and in patients of advanced age, typically age >70 years (14,36-38). Wild-type cancers are distributed widely throughout the colon and rectum however, BRAF mutated cancers are rarely found in left sided colon and rectal cancers but instead are primarily located in the proximal colon (36-39). Additionally BRAF mutant tumors tend to be MSI-high, mucinous histology, serrated and poorly differentiated (14,22,37,40-42). Tran *et al.* (39) further defined BRAF mutant colorectal tumors as a distinct subtype when they delineated a unique pattern of metastatic spread in BRAF mutant cancers, when compared to wild-type tumors. In this study of a cohort of 524 patients with metastatic CRC, 57 (11%) patients were found to harbor a BRAF mutation (55 BRAF^{V600E}, 1 BRAFG593D, 1 BRAFQ609X). Again, female gender, right-sided primary tumor and MSI were statistically significant risk factors associated with BRAF mutant cancers. In patients with BRAF mutations, metastatic spread was more commonly via peritoneal disease (46% *vs.* 24%) or distant lymph node metastasis (53% *vs.* 38%) when compared to BRAF wild-type tumors, and less likely to result in lung metastasis (35% *vs.* 49%). This is clinically relevant as these patients are therefore less likely to undergo metastasectomy as their disease is present in sites not amenable to resection. Not surprisingly, BRAF mutation also conferred poorer overall survival with a median of 10.4 *vs.* 34.7 months. These data suggest that BRAF mutation may serve as a major driver of right-sided tumor biology given the strong association between BRAF mutations and proximal colon cancers and may contribute to the differences in prognosis and metastasis observed between right-sided and left-sided colon cancers.

Figure 2 MAPK signaling pathway in CRC. Mutations in KRAS and BRAF result in constitutive activation of the signaling cascade resulting in uninhibited cellular proliferation and tumor growth. These effects occur downstream of the EGFR receptor, rendering this mutant tumors resistant to anti EGFR therapies. CRC, colorectal cancer; EGFR, epidermal growth factor receptor; MEK, mitogen-activated protein kinase

Table 1 A summary of data: the incidence and interactions of MSI, CIMP and BRAF mutation in CRC

Reference	Cohort (n)	Stage	MSI-high (%)	CIMP-high (%)	BRAF mut (%)
Samowitz *et al.* (22)	911	I-IV	9	27	9.5
Barault *et al.* (23)	582	I-IV	14	17	13
Lee *et al.* (24)	134	II-III	31	14	5
Dahlin *et al.* (25)	604	I-IV	6	8	15
Min *et al.* (26)	245	I-IV	20	14	5
Jover *et al.* (27)	302	I-IV	26	33	21
Lochhead *et al.* (28)	1,253	I-IV	15	17	15

MSI, microsatellite instability; CIMP, CpG island methylation phenotype; CRC, colorectal cancer.
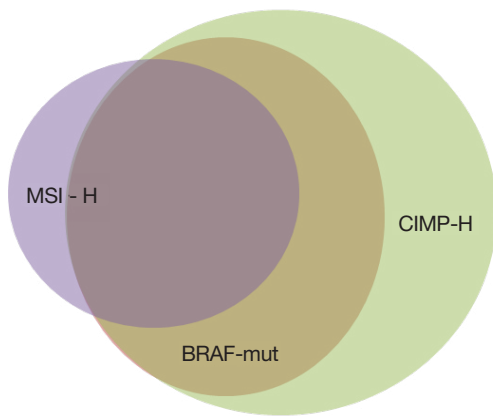
Figure 3 Venn diagram showing the relationship between MSI, CIMP and BRAF mutation in CRC. While these numbers vary greatly depending on the stage of disease, approximately 17% of CRC are MSI-high (MSI-H) (33). Lynch Syndrome accounts for one quarter of these tumors while the rest result from BRAF mutant hypermethylation (34). CIMP-high (CIMP-H) tumors account for approximately 20% of all CRCs with BRAF mutation contributing about one third of these tumors (19,23,35). *Table 1* provides a summary of the data supporting the incidence of these tumor types in CRC. MSI, microsatellite instability; CIMP, CpG island methylation phenotype; CRC, colorectal cancer.

Prognostic and predictive value of BRAF mutation

Overall survival in metastatic CRC has improved significantly due to the development of new chemotherapy and targeted drugs, as well as more liberal use of curative surgical metastasectomies. Despite these advances however, patients with BRAF mutant CRC have low response rates to conventional therapies and poor overall survival. This is true for patients regardless of their stage at the time of diagnosis. Samowitz *et al.* (22) evaluated a large cohort of patients (n=911) with stage I through IV colon cancer. BRAF mutation was seen in 9.3% of all tumors and 52% of MSI high tumors. In this series, the 5-year overall survival of patients with BRAF mutant CRC was significantly lower when compared to wild-type tumors at 47.5% *vs.* 60.7%. The difference was even more pronounced when patients with MSI high tumors were excluded from the analysis, as MSI high tumors are generally associated with a good overall prognosis. They found that in MSS tumors, BRAF mutation was prognostic for poor overall survival on univariate and multivariate analysis with adjustments for clinicopathologic factors including age, stage, and tumor

location. MSI tumors conferred excellent prognosis in this study and BRAF mutation status had no significant effect on 5-year overall survival.

Others have reported similar findings in early stage disease. Roth *et al.* (43) evaluated the prognostic value of KRAS and BRAF mutations in stage II and III colon cancer and found that while BRAF mutation did not predict tumor recurrence, it was prognostic for poor overall survival. This was especially true in MSI-low and MSS tumors (HR =2.2; 95% CI, 1.4-3.4; P=0.0003). Similarly, Fariña-Sarasqueta *et al.* (44) found in a cohort of stage II and III colon cancer, that BRAF mutation was an independent prognostic factor for decreased overall survival in MSS tumors with no difference in overall survival in MSI-high tumors.

Several other studies have demonstrated that the adverse prognosis seen in BRAF mutated CRC is not limited to MSS tumors. Lochhead *et al.* (28) examined the implication of BRAF mutation on overall survival in 1,253 patients with colon and rectal cancers. BRAF mutation was present in 182 (14%) patients, with 55% of those tumors exhibiting MSI-high phenotypes. In this study MSS/BRAF mutant tumors were associated with the highest CRC-specific mortality (HR =2.10; 95% CI, 1.5-2.9; P<0.001). Patients with MSI-high tumors fared better than MSS tumors in general, however MSI-high/BRAF mutant tumors did worse than their MSI-high/BRAF wild-type counterparts with a HR of 0.44 (95% CI, 0.26-0.75; P=0.003) when compared to a HR of 0.26 (95% CI, 0.13-0.52; P<0.001). Likewise, Sinicrope *et al.* (45) found BRAF mutation conferred a worse overall survival in patients with stage III colon cancer regardless of mismatch repair proficiency. However, given the drastic difference in prognosis between MSS/BRAF-mutant and MSI/BRAF-mutant tumors, molecular subtyping alone is an insufficient prognosticator and further underscores that evaluation of mismatch repair proficiency remains critical in the subtyping of CRCs.

Studies in the setting of metastatic disease have further validated these findings. The MRC FOCUS Trial sought to evaluate KRAS and BRAF mutation as prognostic factors in advanced disease. In this study of stage IV CRC, BRAF[V600E] mutation was found to be a negative prognostic marker for overall survival (HR =1.82; 95% CI, 1.36-2.43; P<0.0001), however there was no significant difference in progression free survival (46). To date, BRAF mutation remains the only oncogenic mutation that predicts poor prognosis in metastatic CRC. Tran *et al.* (39) elucidated the impact of BRAF mutation and MSSI in stage IV CRC. In this cohort, BRAF mutation was again found to be a negative prognostic

marker for all patients. More importantly, they reported a strong association between MSI and BRAF mutation and demonstrated poorer overall survival in MSI metastatic CRC suggesting that unlike early stage disease, MSI is a negative prognostic factor in advanced disease and is likely driven by BRAF mutation (39). The poor response to therapy and shorter overall survival of BRAF mutant tumors observed in the studies outline above, are independent of the therapy used and persist over consecutive lines of systemic therapy (47-49).

The role of BRAF mutation status as a predictive molecular marker is less clear. Perhaps the most investigated predictive role of BRAF mutation is as a biomarker of anti-EGFR antibody resistance. KRAS mutation is an established predictive biomarker for anti-EGFR therapy resistance. The KRAS oncogene renders colorectal tumors resistant to anti-EGFR therapies by activating the Ras-Raf-MAPK pathway downstream of EGFR. Similarly, several studies have suggested that BRAF mutation also confers poor outcomes with anti-EGFR therapy through a similar mechanism. Di Nicolantonio et al. (50) and Loupakis et al. (51), in their small cohorts, studied response to anti-EGFR therapy in combination with other chemotherapeutic agent but failed to identify patients with BRAF mutant tumors who responded to anti-EGFR monoclonal antibodies. While other studies have failed studies have failed to show a negative relationship between BRAF mutation and anti-EGFR response, it does appear that BRAF mutation may have a strong predictive role for poor response to cetuximab and should be considered in individualized treatment plans. Richman et al. (46) sought to investigate the predictive implication of BRAF mutation in tumor response to conventional chemotherapeutic agents (irinotecan and oxaliplatin) independent of anti-EGFR therapy in metastatic CRC. BRAF mutation was not found to be a predictive biomarker for irinotecan or oxaliplatin. Patients benefited from the addition of either drug to Fluorouracil in first-line treatment with a slight improvement in progression-free survival but no benefit in overall survival.

Recent data have also suggested that BRAF mutation predicts poor outcomes after metastasectomy. Yaeger et al. (52) described their experience with complete resection of patients with metastatic CRC and noted that patients with BRAF mutations were less likely to undergo metastasectomy (26% vs. 41% at 2 years from diagnosis) due to increased peritoneal spread and decreased liver involvement. Patients with BRAF mutated tumors that were able to undergo complete resection had a trend towards shorter relapse-free survival (7 vs. 11 months) and had a statistically significant shorter overall survival (61% vs. 86% at 2 years) when compared to BRAF wild-type patients undergoing R0 resection for metastatic CRC.

Targeting BRAF in colorectal cancer (CRC)

The BRAF^{V600E} mutation has been widely studied in melanoma. In 2011 vemurafenib, a protein kinase inhibitor of BRAF^{V600E} was approved by the FDA for the treatment of metastatic melanoma after promising results in phase 3 studies with a reasonable safety profile. This made BRAF mutation in CRC an attractive therapeutic target. Unfortunately, the clinical response of single agent BRAF^{V600E} inhibition in early phase studies of metastatic CRC is not as robust as that seen in melanoma (53,54). Efforts have now been focused on identifying mechanisms of early resistance to BRAF^{V600E} inhibition in CRC. Prahallad et al. (55) reported decreased sensitivity to BRAF inhibition in BRAF^{V600E} mutated CRC lines when compared to melanoma cells. Treatment with PLX4032 kinase inhibitor in CRC cells resulted in an increase in EGFR activation due to ERK mediated feedback leading to cellular proliferation. Prahallad and colleagues further elucidated this feedback loop. ERK is phosphorylated and activated in BRAF mutant tumors and negatively regulates EGFR receptor signaling. However, when BRAF mutant CRC cells are treated with vemurafenib, pERK is inhibited resulting in increased EGFR signaling and ultimately cellular proliferation. These findings were later confirmed by Corcoran et al. (56). Notably, this EGFR feedback activation after vemurafenib treatment is not seen in melanoma confirming that BRAF^{V600E} differs functionally between these two cancer types.

Mao et al. (57) have also identified the PI3K/AKT pathway activation as an alternative means of resistance in BRAF mutant CRC. Their study demonstrated that BRAF mutant CRC cell lines had higher levels of PI3K/AKT activation when compared to their melanoma counterparts. Furthermore, the group demonstrated that cell lines with mutations in PTEN or PI3CA showed less growth inhibition when treated with BRAF^{V600E} inhibitor PLX4720 and combination treatment with PI3K inhibitors and PLX 4720 resulted in the growth inhibition and BRAF mutant colorectal cells.

These results have provided a rationale for the use of combination therapy strategies in treating this subset of patients with poor outcomes and no effective treatment

modalities. Early phase studies are ongoing to explore the synergistic effect of BRAF inhibition, anti-EGFR therapy, and PIK3CA inhibition. Corcoran *et al.* (58) reported their phase I/II experience with dabrafenib (D) in combination with MEK inhibitor trametinib (T) in BRAF mutant metastatic CRC. Forty-three patients received combination D+T therapy with 1 patient achieving a prolonged complete response (>22 months), 5 patients (12%) with a partial response, and 22 patients (51%) with stable disease. These data suggest that suppression of the MAPK signaling pathway with combination BRAF and MEK inhibition may be beneficial in a subset of BRAF mutated patients with metastatic CRC and may result in a durable response. Other trials are aimed at investigating "triple combination" therapy. Bendell *et al.* (59) demonstrated that combination therapy with dabrafenib (D), trametinib (T) plus or minus panitumumab (P) anti-EGFR antibody was well tolerated in patients with BRAF mutant metastatic CRC. In the triple therapy arm (D+P+T), 4/6 patients (67%) achieved partial tumor responses, with the remaining 2 patients exhibiting stable disease. In the doublet arm (D+P), 7/8 patients had stable disease, again demonstrating that combination therapy with two or three agents could be administered with acceptable toxicity and showed early evidence of good clinical activity. Given these findings, combination therapy using novel targeted therapeutics and/or traditional cytotoxic agents may lead to better and more durable clinical responses in patients with BRAF mutant colorectal tumors when compared to monotherapy with BRAF^{V600} inhibition.

Conclusions

BRAF mutant tumors represent a discrete subset of CRC characterized by poor overall survival, unique patterns of metastatic spread, and limited response to current chemotherapy and targeted therapies. BRAF has emerged as a key prognostic and predictive biomarker and represents a promising molecular target in the treatment of CRC. To date, monotherapy with vemurafenib and other single agent BRAF^{V600} inhibitors have not produced the desired antitumoral activity and clinical efficacy observed in melanoma due to ERK and/or PIK3CA mediated resistance. Further understanding of the mechanisms of resistance to BRAF inhibition is necessary to develop combination therapeutic strategies which offer the best hope of long term survival in this subset of patients with limited viable therapeutic options.

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