

Clinical implications of *BRAF* mutation test in colorectal cancer

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ABSTRACT

Knowledge about the clinical significance of V-Raf Murine Sarcoma Viral Oncogene Homolog B1 (*BRAF*) mutations in colorectal cancer (CRC) is growing. *BRAF* encodes a protein kinase involved with intracellular signaling and cell division. The gene product is a downstream effector of Kirsten Ras 1(*KRAS*) within the *RAS/RAF/MAPK* cellular signaling pathway. Evidence suggests that *BRAF* mutations, like *KRAS* mutations, result in uncontrolled, non-growth factor-dependent cellular proliferation. Similar to the rationale that *KRAS* mutation precludes effective treatment with anti-EGFR drugs. Recently, *BRAF* mutation testing has been introduced into routine clinical laboratories because its significance has become clearer in terms of effect on pathogenesis of CRC, utility in differentiating sporadic CRC from Lynch syndrome (LS), prognosis, and potential for predicting patient outcome in response to targeted drug therapy. In this review we describe the impact of *BRAF* mutations for these aspects.

Keywords: Colorectal Cancer, *BRAF* mutation, Prognosis value.

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Molecular pathway in colorectal cancer

Colorectal cancer (CRC) is a major medical and public health challenge that develops via a series of genetic and epigenetic changes. These alterations result in the transformation of normal mucosa to a premalignant polyp, and ultimately to a tumor (1-3).

At least three different molecular pathways have been postulated as main players in CRC: chromosomal instability (CIN), Microsatellite

instability (MSI) and CpG island methylator phenotype (CIMP) (4). CIN is the most common cause of genomic instability in CRC and is responsible for approximately 65–70% of sporadic CRC (5).

CIN, or classic adenoma-to-carcinoma pathway, is characterized by an imbalance in chromosome number (aneuploidy), chromosomal genomic amplifications, and a high frequency of LOH, which has been determined through a series of mutations in tumor suppressor genes, such as *APC* and *p53*, and oncogenes, such as *KRAS* (6). The most common genetic alterations are mutations in *APC* and *KRAS* genes (7). A very small percentage of chromosomal instability tumors are inherited and arise secondary to

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germline mutations in the *APC* gene (familial adenomatous polyposis; less than 1% of CRCs) or the *MUTYH* gene (MUTYH-associated polyposis; $\leq 1\%$ of CRCs) (8).

The second pathway, microsatellite instability (MSI), is observed in 15% of CRCs and also most of these tumors are sporadic, in which damaged DNA mismatch repair (MMR) enzymes contribute to acquire copy number variants in repeat sequences of microsatellites. This mechanism is identified by a test for MSI, which categorizes each tumor as MSI-high (MSI-H), MSI-low (MSI-L), or microsatellite stable, based on evaluating the size of multiple microsatellites (9).

The last pathway is characterized by epigenetic alterations, resulting in changes in gene expression or function without changing the DNA sequence of that particular gene (5). For example, methylation of CpG islands in distinct promoter sites can lead to the silencing of critical tumor suppressor genes. The resulting tumors are termed to have the CpG Island Methylator Phenotype, or CIMP (5). CIMP tumors have been closely correlated with mutations in the *BRAF* oncogene (4, 10-12).

Several papers have documented in serrated polyps (sessile serrated adenoma, traditional serrated adenoma, and hyperplastic polyp) a high frequency of *BRAF* mutations, and a low frequency of *KRAS* mutations, and in conventional adenomas, a low frequency of *BRAF* mutations and a high frequency of *KRAS* mutations. This observation provides further data to support the hypothesis that serrated polyps are precursor lesions of CIMP+ CRCs, which have a high frequency of *BRAF* mutations and a low frequency of *KRAS* mutations (13-14). Mutations in the *KRAS* and *BRAF* genes may be observed in *RAS/BRAF/MEK/ERK* pathway (MAPK signaling) (15). Together, these observations result in a growing clinical importance of *BRAF* mutation in CRC patients (16). The *BRAF* gene is composed of 18 exons, and the major common mutation is

found in exon 15 at nucleotide position 1799, accounting for more than 90% of all mutations. This thymine to adenine transversion within codon 600 leads to substitution of valine by glutamic acid at the amino acid level. The other commonly mutated are exon 11, codon 468 and exon 15, codon 596 (17). Recently, *BRAF* mutation testing has been utilized into routine clinical laboratories because of its beneficial operation in differentiating sporadic CRC from hereditary in MSI tumors, determination of clinical prognosis, and prediction of response to drug therapy (18).

Distinguish Lynch syndrome from sporadic CRC

Hereditary non-polyposis colorectal cancer (HNPCC), also called Lynch syndrome, is characterized by MSI and is the most common hereditary colon cancer, accounting for 2-6% of all colorectal cancer burden (19). Available data has provided evidence that, family history alone is unreliable for identifying HNPCC cases. So tumor screening methods such as immunohistochemistry (IHC), genetic testing for mutations and microsatellite instability (MSI) should be considered for identifying individuals with HNPCC.

There are different diagrams for evaluation of LS using MSI, IHC and genetic testing. Every approach has benefits and drawbacks and may depend on provider preferences and institutional resources. Of course each test will miss about 5-15 percent of all HNPCC cases. In this context, accumulative data show that the events leading to HNPCC is an inherited mutation of DNA mismatch repair (*MMR*) gene, mainly *MLH1* or *MSH2*, which account for approximately 90% of the known mutations to date. Subsequent, mutations in *MSH6* account for almost 10% of the cases and bottommost, mutations in *PMS2* have been also reported in a few cases (20-22).

Microsatellites are prepared to instability when mutations are detected in *MMR* genes. Relative to the panel of MSI markers, 80-91% of *MLH1* and *MSH2* mutations and 55-77% of *MSH6* and *PMS2* mutations will be detected by MSI testing. However, MSI in sporadic colorectal cancer is most often associated with hypermethylation of the *MLH1* gene promoter (23). The *BRAF* V600E mutation is often correlated with this sporadic *MLH1* promoter methylation and this mutation has not been detected in tumors that arise from individuals with a germline mutation in *MMR*. Thus, it has been proposed that when IHC reveals absent *MLH1*, evaluated of *BRAF* mutation may avoid unnecessary further genetic testing for identifying tumors as a result of LS. *BRAF* mutation screening may identify HNPCC in MSI-H tumors, although it may not be applicable in the case of *PMS2* mutation carriers (24-28).

According to a study by Loughrey *et al.*, the *BRAF* V600E mutation has been reported as a germline mutation in 17 of 40 (42%) tumours showing loss of *MLH1* protein expression by immunohistochemistry, but only in patients with sporadic CRC. The authors recommend the incorporation of *BRAF* V600E mutation testing into the laboratory algorithm for pre-screening patients with suspected HNPCC, whose CRCs show loss of expression of *MLH1* (28): If a *BRAF* V600E mutation is present, no further testing for HNPCC would be warranted. Also another study showed that detection of the *BRAF* V600E mutation in a colorectal MSI-H tumor rules out of the presence of a germline mutation in either the *MLH1* or *MSH2* gene (25). The result of this study showed that the *BRAF*-V600E hotspot mutation was found in 40% (82/206) of the sporadic MSI-H tumors analysed, but in none of the 111 tested HNPCC tumors. Thus screening CRC for *BRAF* V600E mutation is a reliable, fast, and low cost strategy that simplifies genetic testing for HNPCC (25).

The concurrent use of MSI testing, *MMR* protein IHC and *BRAF* mutation analysis would detect almost all *MMR*-deficient CRC (29). A suggested algorithm incorporating somatic *BRAF* V600E mutation testing of tumor tissue into the investigation of suspected HNPCC is shown in Fig. 1 and in Fig. 2.

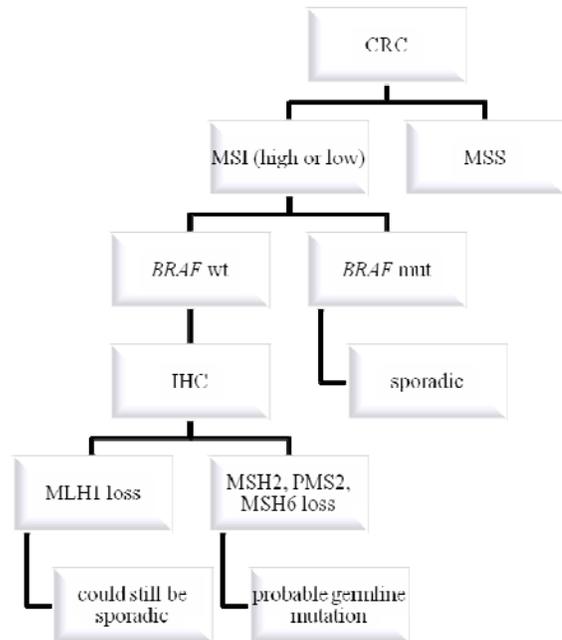


Figure 1. Algorithm for genetic testing in colorectal cancer, following the microsatellite instability (MSI) route, when the samples are first tested for microsatellite instability, next for mutated *BRAF*, and finally for expression of mismatch repair enzymes by immunohistochemistry (IHC).

Predicting patient outcome in response to targeted drug therapy

Recently, papers demonstrated that information about *BRAF* mutation status, like for *KRAS*, is useful to help select efficacious therapy, especially when selecting systemic chemotherapy (30). In this context, many papers showed that, the patients harboring *KRAS* mutation do not respond to anti-epidermal growth factor receptor (EGFR)

therapies, but many patients with wild type *KRAS* also do not respond to this therapy (4).

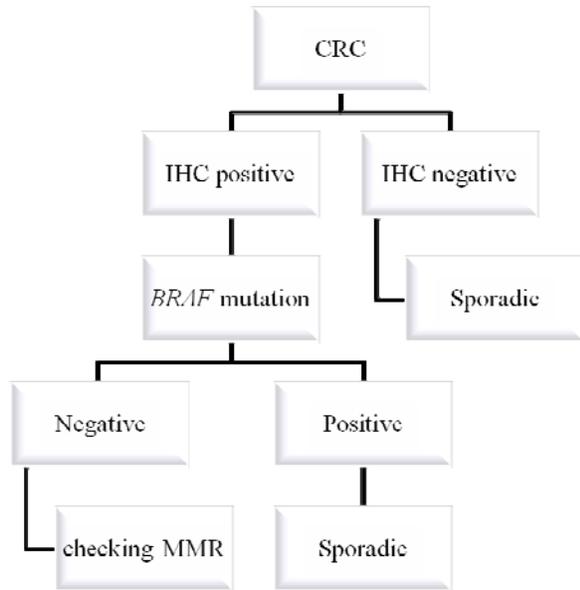


Figure 2. Algorithm for genetic testing in colorectal cancer when the samples are first tested for expression of mismatch repair enzymes by immunohistochemistry (IHC), next for mutated BRAF, and finally checking MMR in full sequence

It has been suggested that other mutations, such as *BRAF* and *PIK3CA*, have a critical role in those cases (31). *BRAF* testing is suggested in CRCs that are negative for *KRAS* mutation when the patient is being pondered for anti-EGFR therapy. Colucci et al. in 2010 showed that the efficacy of anti-EGFR mAb is confined to patients with wild-type *KRAS*, whereas no mutations in any of the patients were detected in the *BRAF* gene (32). The efficacy of *BRAF* mutation on cetuximab or panitumumab response was also evaluated by cellular models of CRC (30). According to an Italian paper, 53% of the patients (110 out of 209) were considered as potentially non-responders to anti-EGFR therapy because of *KRAS*, *BRAF* or *PI3K* mutations (33).

It is now recognized that anti-EGFR mAb therapy should only be used in patients whose tumors express wild-type *KRAS*. Furthermore,

BRAF, *PTEN*, and *PI3K* are emerging as future potential predictive markers of response. However, further clinical studies are warranted to define the role of these biomarkers (34). An algorithm that includes testing for *KRAS* and *BRAF* mutation for the selection of patients for anti-EGFR therapy is shown in Fig. 3.

Predicting prognosis of CRC

The last potential use of *BRAF* mutation testing is for prognosis of CRC (18). In an interesting paper, Kalady et al. documented that *BRAF* mutation is associated with distinct clinical characteristics as mutant tumors were characterized by female sex, advanced age, proximal colon location, poor differentiation, MSI, and importantly, worse clinical prognosis for the patient (4).

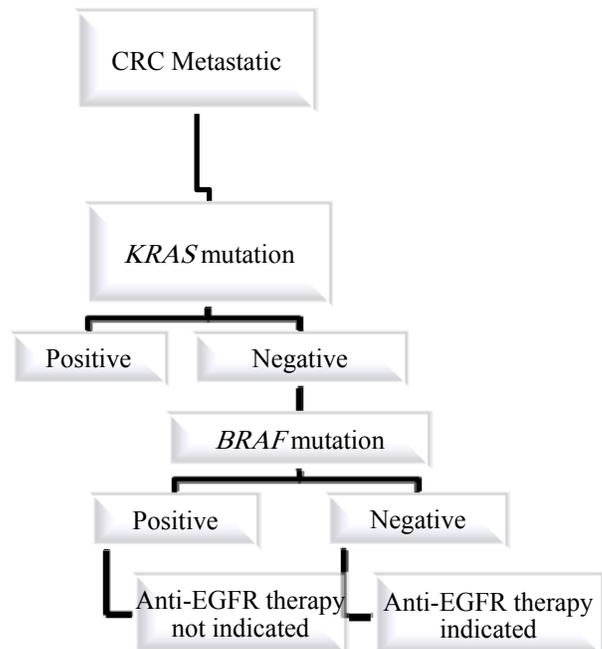


Figure 3. Algorithm for genetic testing in colorectal cancer when the patient is a candidate for Anti-EGFR therapy.

They showed that *BRAF* mutation was independently associated with decreased overall survival. Many studies suggest that the association between *BRAF* mutation and CRC survival may differ by some factors. Phipps et al. documented that poor clinical prognosis associated with *BRAF* mutation was limited to cases diagnosed at ages <50, and in another study Samowitz et al. reported that this poor clinical outcome in CRC were microsatellite stable and not MSI-H cases (35-36). Farin~ a-Sarasqueta et al. in 2010 reported that the V600E *BRAF* mutation confers a worse prognosis in stage II and stage III colon cancer patients independently of disease phase and therapy (37). In one interesting paper, Teng et al. in 2012 showed that *BRAF* mutation is an independent prognostic biomarker in patients with liver metastases after metastasectomy (38). Ogino et al. suggest that, the worst prognosis associated with *BRAF* mutation may in part be disannulled by a high CIMP status and the good prognosis associated with MSI-high status is partly weakened by a mutated *BRAF* status (39).

Status of KRAS and BRAF mutations in relation with CRC in Iran

There are few data on *BRAF* and *KRAS* mutation in Iran (40- 41). In one valuable study, tumor samples from 182 Iranian colorectal cancer patients (170 sporadic cases and 12 HNPCC cases) were screened for *KRAS* mutations at codons 12, 13 and 61 by sequencing analysis (40). *KRAS* mutations were observed in 68/182 (37.4%) cases, which is slightly lower as compared to the outcome of a study on an Italian population (33). Mutation frequencies were similar in HNPCC-associated, sporadic MSI-H and sporadic microsatellite-stable (MSS) tumors (40). Another study was done by Shemirani *et al.*

and showed that probably the profile of *KRAS* mutations in tumors is not entirely compatible with the pattern of mutations in polyps (41). Montazer Haghighi *et al.* investigated 78 patients and determined with the Pentaplex Panel of mononucleotide repeats that 21 patients (26.9%) had tumors that were MSI-H, 11 patients (14.1%) were MSI-L and 46 patients (59%) were MSS. There were no statistically significant different between MSI-H, MSI-L and MSS regarding clinical features, pathology or family history of cancer in the patients (42). However, Naghibalhosseini *et al.* reported high frequency of genes promoter methylation, but lack of *BRAF* mutation among 110 unselected of sporadic patients (43). It is conclude that studies are warranted to determine the prevalence of *BRAF* mutation in different site of Iran to examine their impact on prognosis and response to targeted treatment. Iran is vast country and has people from various ethnic backgrounds. Therefore, we suggest to do clinical studies on the correlation between *BRAF* mutation and ethnic background. The results will show if the prevalence of *BRAF* mutations in CRC differs by ethnic background and also whether ethnic background has influence on clinical prognosis or response to drug treatment.

Summary

In terms of prognosis, an emerging body of literature describes worse clinical prognosis and decreased response to therapy for patients with *BRAF* mutant tumors. *BRAF* mutation has been reported to have a worse prognosis in MSS tumors, but there is little information regarding the effect of *BRAF* mutations on MSI-H tumors. In addition to prognosis, *BRAF* mutation could have clinical treatment implications. Similar to patients with *KRAS* mutations, patients with metastatic tumors that are being considered for anti-EGFR therapies should be tested for *BRAF*

mutations as well. Because *KRAS* is more frequently mutated than *BRAF*, first-line testing should be done for *KRAS*. If the tumor is *KRAS* wild type, then genotyping *BRAF* should be considered. We propose studies in CRC in Iran on the clinical impact of *KRAS* and *BRAF* mutation, especially considering ethnic background.

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