

## Lack of BRAFV600E mutation in stage I and II of colorectal cancer

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### ABSTRACT

**Aim:** We aimed to explore the frequency of BRAFV600E mutation in Iranian patients with colorectal cancer (CRC) as well as its association with clinic pathological characteristic of patients.

**Background:** CRC is the third leading cause of cancer related death. There is a growing body of data showing the association of BRAFV600E mutation with malignant transformation and clinical outcome of different tumors, including CRC. These findings suggest that BRAFV600E mutation can be used as diagnostic and/or prognostic biomarker for management of cancer patients.

**Patients and methods:** A total of 85 patients with sporadic tumor were recruited. BRAFV600E mutation was investigated using sequencing of extracted DNAs from formalin-fixed paraffin-embedded (FFPE) tumor tissues. Electropherograms were analyzed using Laser-gene 6 software.

**Results:** More than 95% of patients were in stage I and II and none of them were in stage IV. Patients were mostly below 55 years old and tumors were dominantly located in the distal colon. Of note, no BRAFV600E mutations were detected in our population.

**Conclusion:** Our results showed no V600E mutation in the BRAF gene in stage I and II of CRC patients. Further studies in multi-center settings are warranted to examine the prognostic and/or predictive value of this marker in different stages of colorectal cancer patients.

**Keywords:** BRAFV600E mutation, CRC, DNA sequencing.

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### Introduction

Colorectal cancer (CRC) is the third-most-common form of cancer in the world (1). Several genetic mutations have been reported to be associated with the development and progression of this disease, including V-Raf Murine Sarcoma

Viral Oncogene Homolog B1 (BRAF). BRAF is one of the main genes, involved in the progression of several malignancies, including CRC (2, 3). This gene encodes cytoplasmic serine-threonine kinase that mediates intracellular signaling through the RAS/RAF/MAPK pathway (4). The mitogen activated protein kinase (MAPK) pathway is one of the most critical pathways in the regulation of

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cancer cell proliferation and survival (5). Oncogenic mutations in BRAF cause constitutive activation of MAPK pathway (6). More than 80% of mutation are found in exon 15 at nucleotide position 1799, causing thymine to adenine transversion within codon 600 leading to substitution of valine by glutamic acid at protein level (2, 7). Activating BRAF mutations have been identified in 4% to 12% of unselected colorectal tumors in all Dukes' stages (8-13). Moreover, this mutation has been reported in several forms of premalignant lesions, such as adenomas, hyperplastic polyps, and serrated adenomas (5-6), as well as in aberrant crypt foci (14, 15). BRAF gene is usually present as wild type in hereditary non-polyposis colorectal cancer (HNPCC), whereas the BRAFV600E mutation is often found in the sporadic CRC (16-18). Several studies have shown that BRAFV600E mutation is associated with survival in metastatic CRC patients, suggesting its prognostic value (19). BRAFV600E mutation is also being suggested as a predictive biomarker in response to anti-EGFR drug therapy. In particular, patients harboring this mutation were resistant to Cetuximab and panitumumab drugs (19). Due to the crucial role of BRAFV600E mutation in diagnostic, therapeutic and prognostic fields, in the present study, we further investigate the prognostic value of this marker with clinical outcome of 85 CRC patients.

## Patients and Methods

### Patients

This retrospective cross sectional study was performed on 85 randomly selected patients with colorectal cancer, who had undergone surgical resection of adenocarcinoma and referred to the gastroenterology and liver disease research center of Shahid Beheshti University of Medical Sciences (Tehran, Iran) from June 2013 to December 2014. Patients with Familial

Adenomatous Polyposis coli (FAP) or hereditary non-polyposis CRC (HNPCC) were excluded. Informed consent was obtained from all the patients, or their relatives. Demographic and clinical information of the patients were obtained; including: age, sex, tumor location, tumor grade, smoking status and alcohol consumption. Tumor tissue specimens were harvested from tumor mass after surgical resection.

### Deoxyribonucleic acid (DNA) extraction

DNA was extracted from paraffin-embedded tissues using the Qiagen kit. The quality of the extracted DNA was assessed by NanoDrop spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA).

### DNA sequencing

The 160 bp DNA fragment of the exon 15 of BRAF gene was amplified using specific primers (forward 5'GCTTGCTCTGATAGGAAAATGAGATC3' and reverse 5'ATCCAGACAACTGTTCAAAGTATG3'), as described previously (20). To observe the digested fragments, the PCR products were separated in 2% agarose gel and stained with ethidium bromide. Direct sequencing was performed, using fluorescent dideoxy on ABI sequencing 3130 XL, according to manufacturer instructions. Samples were then subjected to direct sequencing of single strand PCR product using Big Dye Terminator v 3cycle sequencing kit and the ABI 3130XL genetic analyzer (Applied Biosystems). The electropherograms were processed using Lasergene software version 6 (DNA star).

### Statistical analysis

Statistical analysis was performed using the SPSS software program for Windows, Release 13.0.0 (SPSS Inc., Chicago, IL). Comparison of variables was performed using Pearson's Chi-

square test, Fisher's exact test, or the Mann-Whitney U test, depending on the nature of the data. Two-tailed  $p < 0.05$  was considered statistically significant.

## Results

### *Demographic and Clinical characteristic of population*

A total of 85 colorectal cancer patients were enrolled in the present study. Demographic and clinicopathological features of patients are summarized in Table 1. Most of patients were male, non-smokers and non-alcoholic. Age distribution was more frequent in younger patients (52.9%) and tumors locations were dominant in distal colon (54.1%), compared to proximal colon (45.8%). More than 95% of patients were in stage I and II, while there were no patients in stage IV.

### *BRAFV600E mutation status of the patients*

To evaluate the mutation status of our patients for BRAFV600E, direct sequencing was carried out in genomic DNA extracted from FFPE of all cases, using the fluorescent dideoxy method. Our analyses illustrated that none of patients had BRAFV600E mutation.

## Discussion

Colorectal cancer is a heterogeneous disease with multiple underlying genetic mutations, leading to the development of this disease (21-25). Mutations in BRAF gene have been documented to be involved in malignant transformation of tumor tissue. Several studies have reported the frequency of BRAFV600E mutation in CRC between 0 and 23% in different populations. In particular, Fariña-Sarasqueta and colleagues investigated the value of BRAF, microsatellite instability (MSI) and KRAS mutations on clinical outcome of 106 stage II colon carcinoma patients undergone surgery and 258 stage III patients treated with 5-fluorouracil chemotherapy. They showed that the V600E BRAF mutation were associated with poor prognosis in CRC patients (26). Another study by Shaukate et al., examined the correlation of BRAFV600E mutation in 63 intervals versus 131 non-interval colorectal cancer patients. They found no association between this mutation and interval cancers, although they suggested its value as a marker of poor prognosis, particularly in microsatellite stable cancers (27). There is a growing body of evidence reporting the

**Table 1.** Characteristic of colorectal cancer patients

Characteristic		Number of patients	Percentage
Sex	Male	48	56.4
	Female	37	43.5
Age at diagnosis	< 50 year	45	52.9
	≥ 50 year	40	47
Tumor Site	Proximal	39	45.8
	Distal	46	54.1
Tumor Grade	I	48	56.4
	II	33	38.8
	III	4	4.7
	IV	0	0
Smoking	Current user	11	12.9
	Never user	62	72.9
	Previous user	12	14.1
Alcohol	Current user	4	4.7
	Never user	75	88.2
	Previous user	5	5.8

rate of BRAF mutation in CRC patients, such as in Switzerland (19.7%) (28), Netherland (18.7%) (26), USA (21.8%) (27), Italy (14.9%) (29), France (4.3%) (30), and Belgium (4.7%) (31). A low incidence of BRAF mutation in CRC was observed in Asians e.g. Thailand (0%) (32), Saudi Arabia (2.5%) (33), China (4.9%) (34), Taiwan (1%) (35), and Japan (4.7%) (36). This can be explained at least in part by ethnicity. Thus, we conducted a cross sectional retrospective study to determine the rate of BRAFV600E mutation in an Iranian population with colorectal cancer. We also investigated the association of this mutation with clinicopathological features of cases, including tumor location, grade, and demographic data such as age, gender, smoking and alcohol consumption. Our data showed enrolled patients in this study didn't have any mutation in BRAF gene. In line of our observation Naghibalhossaini et al. showed the absence of BRAF mutation among the Iranian population (24). Additionally Brim et al., reported a very low rate of mutation in BRAF among CRC patients in Iran (2 %) (22).

On the other hand, a recent study revealed that BRAF mutation is more common in older (>60yr) and female patients (37). However, most of our cases were below 55 years old, which might explain the lack of this mutation in our cases. Moreover, some studies reported a relationship between the history of smoking in CRC patients with BRAFV600E mutation (38-40). Their results showed a negative correlation between BRAFV600E mutation and alcohol intake. Our patients participated in this study were non-smokers and non-alcoholic. This might be another reason for the lack of BRAF mutation in our cases. In addition, this mutation was found mostly in patients with stage III, although our subjects were in stage I and II. This could explain that BRAF mutation might be present in late stages.

In conclusion, our data showed the lack of BRAF mutation in stage I and II of CRC patients, supporting further studies in a larger population

and multi center setting to evaluate the prognostic and/or predictive value of BRAF mutation in CRC patients.

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