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EVALUATION OF MUTATIONS IN KRAS AND BRAF GENES IN IRANIAN POPULATION WITH DIFFUSE GASTRIC CANCER

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Abstract – *Objective:* *RAS proteins control signaling pathways which are the main regulators of the normal cell growth and malignant transformation cells. The point mutations in the KRAS gene are current event in numerous human cancers including pancreatic, lung, colon, and breast cancer. BRAF protein is located in the downstream of KRAS and it is revealed to be somatically mutated in various human cancers. KRAS and BRAF mutated genes have a vital role in the establishment and development of tumors. Since there is a little information about BRAF and KRAS mutations in gastric cancer, the present study has been designed.*

Materials and Methods: *We assessed 31 cases that experienced gastrectomy for diffuse gastric cancer, according to the histopathological criteria confirmed by pathologist. The patients were hospitalized in the Al-Zahra hospital in the Isfahan province (central Iran) and in the Alaa Cancer Control Center from 2011 to 2016. The DNA sequencing was completed by amplification exon 2 of KRAS and exon 15 of BRAF genes.*

Results: *According to the electropherogram of DNA sequencing we did not find any alterations in the codon 12 and 13 of KRAS and codon 600 of BRAF genes.*

Conclusions: *Our results demonstrated that there was not association between codon 12, 13 of KRAS gene, and codon 600 of the BRAF gene in patients with diffuse gastric cancer in the Iranian population. The results of this study reveal that MAP kinas signaling pathway does not play any role in diffuse gastric cancer in the Iranian patients. Furthermore, the study suggested that other genes are probably involved in this cancer. More evidence is needed in a larger sample size to confirm these results.*

KEYWORDS: *KRAS, BRAF, Gastric cancer, Mutation.*

INTRODUCTION

Gastric cancer (GC) is the fourth most common cancer and the second cancer related death in the world[1]. According to the Lauren classification, gastric cancer is divided into three forms, included intestinal (54%), diffuse (32%) and indeterminate type (15%)[2]. Diffuse gastric cancer (DGC) has late-onset manifestations and a highly invasive state with poor prognosis[3].

The molecular heterozygosity in the GC may have a potential effect on the targeted therapy. The study of the genetic mutations is a major element in the modern era in the treatment of cancer, based on personalized medicine. In the past years, our awareness on some of these mutations and their predictive potential has been revolutionized in the treatment of various malignancies by improving clinical outcomes and patient care[4,5].

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The mitogen-activated protein kinase (MAPK) pathway is a signaling cascade that was involved in the regulation of cellular process such as cell proliferation, differentiation, apoptosis, and survival. The dysfunction of this pathway results in the occurrence and cancers progression particularly due to RAS mutations that are documented as an oncogene in all cancers. The most frequently mutated gene is KRAS with 20% and is followed by NRAS and HRAS with 8% and 3.3% respectively. KRAS mutations revealed in gastric cancer were involved mainly in 25% of microsatellite-instable (MSI) subtypes by The Cancer Genome Atlas Research Network[6]. Also, the RAS proteins are involved in the phosphatidylinositol-3-kinase (PI-3K) pathway[7].

BRAF and KRAS are two main members in the signaling pathway of the epidermal growth factor receptors whose mutations have a predictive value in patients with metastatic colorectal cancer for the treatment with anti-EGFR drugs[8]. Anti-EGFR agents such as cetuximab and panitumumab are used for patients with KRAS wild type in metastatic colorectal cancer, but the patients with mutations in the KRAS gene are resistant to these drugs[9,10]. However, only 40-60% of patients without mutations in the KRAS gene respond to EGFR-based treatments. Therefore, it is very important to identify other molecular changes such as BRAF mutations that may have an effect on anti-EGFR therapy. It seems that determining the prevalence of KRAS/BRAF mutations in gastric cancer patients from different geographical areas is necessary for the clinical development of the treatment based on EGFR[11].

The identification of the molecular markers can provide insights into the pathogenic process and optimize cancer treatments based on personalized medicine. According to the above-mentioned analysis, the aim of this study was to investigate the somatic mutations in KRAS and BRAF genes among DGC patients of Iran.

MATERIALS AND METHODS

Patients and Sampling

In this study we analyzed 31 patients with diffuse gastric cancer using clinical criteria and histological features confirmed by pathologists. The cases were selected among GC patients referred to the Al-Zahra Hospital (a referral hospital in Isfahan province, in central Iran), and Alaa Cancer Control Center (a charity-based foundation in Isfahan) from 2011 to 2016. The total specimens were formalin fixed-paraffin embedded (FFPE) tumor tissues. FFPE tumor samples were cut to 5-10 µm thickness sections and the deparaffinization was performed by the Xylene; then, the DNA extraction was done using a phenol chloroform method. The informed consent form was filled by all patients or their families. Our study was approved by the Review Board of Isfahan University of Medical Sciences.

DNA Sequencing Analysis

The obtained DNA samples were used for the identification of the mutations in KRAS and BRAF genes. The DNA amplification of these genes was performed by Polymerase Chain Reaction (PCR) method. Due to the low quality of the DNA, two samples set aside, and the remained samples were amplified by specific primers designed by the authors (Table 1) in the exon/intron boundaries of the exon 2 of KRAS with 351 bp length of PCR product and in the exon 15 of BRAF genes with 246 bp length of PCR product. The DNA direct sequencing was done through the PCR product of each reaction by ABI 3130XL capillary sequencing platform (Applied Biosystems/Life Technologies, Carlsbad, CA, USA). The sequences were obtained as electropherograms and evaluated using the DNASIS MAX V3 software.

RESULTS

Clinical and Epidemiological Analysis

Of the 31 under-studied patients, 19 were male and 12 were female. 24 of them were over 50 years old, and 7 were less than 50 years old. The mean age at diagnosis in the patients was 57.6 and 60.83 in male and female, respectively.

In 32% of cases, the tumors were in stages I and II, while 68% of cases were in stages III and IV. The histopathological types of tumors reported were the signet ring cell carcinoma (SRCC) in 22 cases and poorly differentiated adenocarcinoma in 9 cases (Table 2).

Sequencing Analysis

Except for two samples that had low DNA quality and were excluded, all the coding exons and flank-

TABLE 1. KRAS and BRAF primers.

Gene	*Forward primers*	*Revers primers*	*Exon*	*Length*
KRAS	ATACACGTCTGCAGTCAACTGG	GTATCAAAGAATGGTCCTGCAC	2	351 bp
BRAF	CATAATGCTTGCTCTGATAGG	CTAGTAACTCAGCAGCATCTCAG	15	246 bp

TABLE 2. Sex, age of diagnosis, stage, and histopathological type of patients.

Sex	Age of diagnosis	Stage	Histopathological type
M	52	IIIA	SRCC
M	48	IIIB	SRCC
F	64	II	PDA
F	65	IB	SRCC
M	68	I	SRCC
M	49	IIIA	PDA
F	75	IIIC	SRCC
F	47	II	SRCC
F	63	IV	SRCC
M	49	IIIA	SRCC
M	53	II	SRCC
F	70	IIIC	PDA
F	62	II	PDA
F	53	IIIC	SRCC
M	52	II	SRCC
M	62	IIIA	PDA
M	40	IIIB	SRCC
F	70	IIIC	PDA
M	57	IIIB	SRCC
M	61	IV	SRCC
M	54	IV	SRCC
F	65	IB	SRCC
F	53	IIIC	SRCC
M	80	II	PDA
M	78	IIIC	SRCC
M	51	IIIA	SRCC
M	62	IIIC	SRCC
M	40	IV	PDA
F	43	I	SRCC
M	68	IIIA	PDA
M	71	IV	SRCC

M: Male, F: Female, SRCC: Signet ring cell carcinoma, PDA: Poorly differentiated adenocarcinoma.

ing intronic regions of the exons 2 of KRAS and exon 15 of BRAF genes were successfully amplified by PCR in all patients with 351 bp length of PCR product for KRAS and 246 bp length for BRAF genes (Figure 1).

The DNA sequencing of the amplified PCR products does not show any alteration in KRAS and BRAF genes of the tumor samples (Figure 2).

DISCUSSION

The management approach of GC is challenged in the current clinical setting with various responses to systemic treatments, which may be due to the heterogeneity in the intra and inter tumors. Molecular heterozygosity in a GC might potentially have an effect on the targeted therapy[12]. Due to uncertainty associated with the prediction of the response to treatment and significant toxicity in relation to systemic chemotherapy, the predictive biomarkers can play a role in the direction of treatment.

Recent advances in targeted therapy reveals improvements in the use of trastuzumab in the treatment of metastatic gastroesophageal carcinoma expressing HER2 that was approved in the ToGA clinical trial[13]. Satoh et al[14], in 2014 evaluated the efficacy of systematic treatments with the combination of paclitaxel ± lapatinib and reported no benefit to the overall survival and progression-free survival in gastric cancer. Also, the TRIO-013/LOGIC randomized trial phase III represented an addition of lapatinib to capecitabine and the oxaliplatin did not increase overall survival in cases with HER2-amplified gastroesophageal adenocarcinoma[15]. These results may be due to the lack of response to anti-EGFR drugs and mutations in genes such as KRAS and BRAF. KRAS and BRAF are two main members of the EGFR signaling pathway, whose mutations have a predictive value in patients with metastatic colorectal cancer for the treatment with drugs such as panitumumab and cetuximab that target EGFR[16]. Harari and Peyssonnaux[17,18] have shown that the gene mutation in EGFR signaling proteins such as KRAS and BRAF, are essential factors in the evaluation of the resistance to anti-EGFR drugs.

The frequency of KRAS mutations is different throughout the world. The BRAF gene is a protein that is part of the Ras-Raf-MEK-ERK or MAPK signaling pathway[19]. Activating this pathway leads to cell growth and proliferation. EGFR signaling plays an important role in the development of cancers such as colorectal. Bos et al[20], first reported KRAS muta-

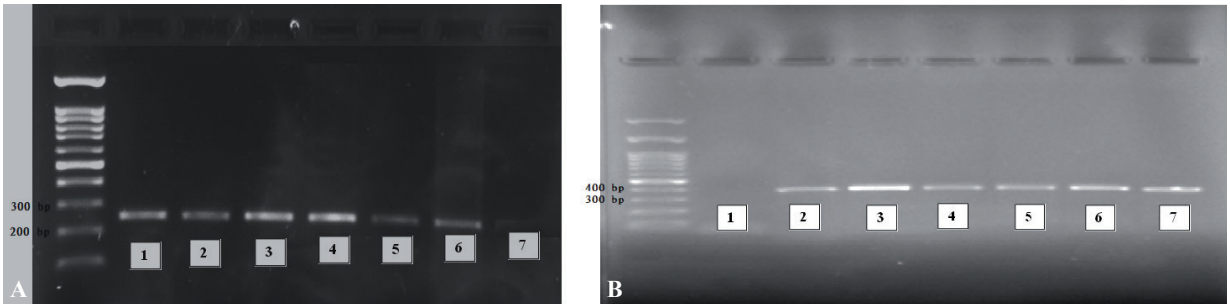

Fig. 1. Gel electrophoresis of KRAS and BRAF genes. **A**, Exon 15 of BRAF gene with 246 bp length. Number 1-6=samples, number 7=negative control. **B**, Exon 2 of KRAS with 351 bp length. Number 1=negative control, number 2-7=samples.

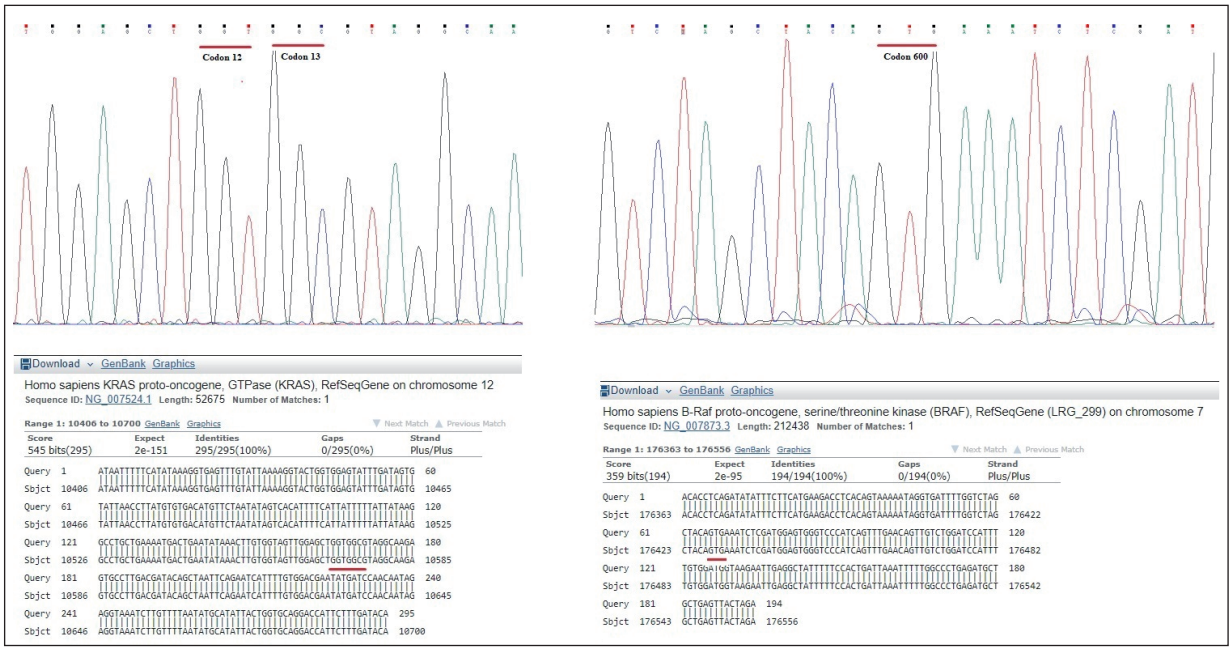

Fig. 2. Electropherogram and BLAST results of KRAS and BRAF genes. **A**, Electropherogram and BLAST results of KRAS gene in exon 2 representative of normal sequence. **B**, Electropherogram and BLAST results of BRAF gene in exon 15 representative of a normal sequence.

tions in gastric cancer in a patient in 1986. Since then, many studies have been conducted on KRAS mutations in gastric cancer, most of which are in patients with diseases in Asia and few of them have been performed on the tumor[21,22]. The previous studies reported that most mutations in the KRAS gene occur in the codons 12 and 13 (Exon 2) of this gene. All studies focus on the mutation status in KRAS codons 12 and 13 in a variety of ways. The mean mutation rate for all gastric cancer cohorts was 6.5%[23]. In the Takahashi et al[24] study on the investigation of the features of clinicopathology and RAS mutations in advanced stomach cancer it was reported that the most common mutations in the KRAS gene were G12D substitution in codon 12 and G13D substitution in codon 13.

In patients with colorectal cancer, the advantage of using EGFR inhibitors is limited to patients whose KRAS is wild-type[16]. A small number of studies[25,26] have examined the status of mutations in the BRAF gene in gastric cancer, and its prevalence has been reported to be 0% to 11%. BRAF gene mutations occur in some human cancers, including colorectal cancer, malignant melanoma, thyroid papillary carcinoma, Non-Hodgkin lymphoma, and lung adenocarcinoma while the V600E mutation for 80% of BRAF mutations and V600K mutations were reported in about 20% of BRAF mutations. Other less common mutations include V600R and V600D mutations[27]. Al-Shamsi et al[28], investigated the mutations in KRAS, NRAS, BRAF, PIK3CA, TP53, and APC genes in colorectal cancer patients and reported that patients with BRAF mutations respond negatively to anti-EGFR medications.

The frequency of mutations in the KRAS and BRAF genes in gastric cancer differs among the population. It seems that determining the prevalence of KRAS/BRAF mutations in large gastric cancer patients from different geographical areas is necessary for the clinical development of treatment based on EGFR. Iran is a large country with a population of about 80 million; however, there isn't available information about the role of KRAS and BRAF mutations in diffuse gastric cancer. On the other hand, obtaining information about mutations in these genes in connection to the response to anti-EGFR drugs seems to be necessary.

We investigated the mutations of KRAS and BRAF genes by Polymerase Chain Reaction (PCR) in tumor tissue samples of patients with diffuse gastric cancer in order to obtain information about these genes as the predictive markers of the response to anti-EGFR treatment. The results of this study failed to show any association between mutations in codon 12 and 13 (Exon 2) of KRAS gene and codon 600 of BRAF gene with diffuse gastric cancer.

CONCLUSIONS

The MAP kinas signaling pathway does not play an important role in diffuse gastric cancer in Iranian patients. Moreover, further evidence is required in other studies with larger sample size to confirm these results. On the other hand, other genes suggested in this study are probably involved in this cancer.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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