RESEARCH ARTICLE

Evaluation of KRAS Gene Mutations in Metastatic Colorectal Cancer Patients in Kermanshah Province

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Abstract

Background: Worldwide, colorectal cancer (CRC) is reported to be the fourth most common cancer in men and the third most common in women. KRAS is a proto-oncogene located on the short arm of chromosome 12. The aim of this study was to evaluate the KRAS oncogene and its relationship it with clinicopathologic features in 33 Kurdish patients. Materials and Methods: Metastatic CRC between 2012 and 2016 that came to Imam Reza hospital, Kermanshah province, Iran, were analysed for KRAS mutations using allele specific PCR primers and pyrosequencing. Correlations between variables was analyzed in PASW SPSS and overall survival curves were plotted in Graph Pad prism 5. Results: The mean age for them at diagnosis was 51.5±12.6 years (range, 22-76 years) . Among the 33 patients that were sequenced, 12 samples in the KRAS gene had a nucleotide change, 11 in codon 12 and 1 in codon 13.There was no significant relationship between the mutation and clinical and pathological aspects of the disease. Conclusions: Knowledge of the KRAS status can help in decision-making to treat metastatic colorectal cancer patients more efficiently and increase survival. However, many Kurdish people due to economic problems are not able to do this valuable genetic test. In addition, we need more careful research of KRAS oncogene at the molecular level in young populations with more patients.

Keywords: Colorectal cancer - KRAS - mutation - PCR - wild type

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Introduction

Colorectal cancer (CRC) is the fourth most common cancer in men and the third most common in women (Madani et al., 2015).With advances in the understanding of the biology and genetics of CRC , diagnostic biomarkers that may predict the existence or future presence of cancer or a hereditary condition, and prognostic and treatment biomarkers that may direct the approach to therapy have been developed (Carethers , 2015).

Cetuximab (Erbitux®) is a monoclonal antibody used widely in the targeted treatment of metastatic CRC(Vecchione et al ., 2011).KRAS is a proto-oncogene located on the short arm of chromosome 12, encodes the protein KRAS, a GTPase involved in cell division, differentiation and apoptosis(Dobre et al ., 2015).It has been frequently reported that patients having KRAS mutations show no significant response to moAb treatment (Bando et al ., 2011).

KRAS mutations are found in approximately 35-45% of CRCs (Van Cutsemet al., 2009) .The Ras/Raf/MEK/ERK kinase cascade is involved in the control of cell proliferation, cellsurvival and invasion in CRC cancer cells (Perkins et al., 2014).However, the clinical significance of KRAS mutation as a prognostic marker is controversial. Some studies reported no association with survival, whereas others suggested that patients withKRASmutated tumors have poorer outcome for any mutation subtype (Rosty et al., 2013; Yoon et al., 2014). The aim of this study was to evaluate the KRAS oncogene and relationship it with clinicopathologic features in 33 Kurdish patients.

Materials and Methods

Patients and Methods

The study did on 33 KRAS gene of patients with metastatic CRC between 2012 and 2016 that they came to Imam Reza hospital, Kermanshah province, Iran.

Method for KRAS Mutation

DNA extracted by FFPE Q1AGEN kit and KRAS analyzed using allele specific PCR primers and pyrosequencing. The results have been double checked by high resolution melting analysis. Detection limit of these assay are five copies of mutation in whole

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genome. Mutation screened for: p.Gly12Asp (c.35G>A), p.Gly12Ala (c.35G>C), p.Gly12Val (c.35G>T), p.Gly12Ser (c.34G>A), p.Gly12Arg (c.34G>C), p. Gly12Cys (c.34G>T), p. Gly13Asp (c.38G>A).

Statistical Analysis

The correlation of between the variables was analyzed in PASW SPSS (version 19) with Chi-square test that $p<0.05$ was statistically significant. Curve of the OS was plotted by Kaplan-Meier plot in Graph Pad prism 5 Software in 2-year period.

Results

The mean age for them at diagnosis was 51.48±12.6 years (range, 22-76 years) that 26 patients (79%) were male and 7 patients (21%) were female.Of 33 patients, 21(64), 8(24), 2(6), 1(3) and 1(3) had metastasis to liver, lung, lung + liver, peritoneum and pelvis, respectively. In all of patients, kind of pathology in 32 patients (97%) was invasive adenocarcinoma and 1 patient (12.1%) was mucinous adenocarcinoma.Location of tumor in patients was: rectum (45%), rectosigmoid (33%), sigmoid (16%), descending colon (3%) and transverse colon (3%) (Table 1).

Among the 33 patients that were sequenced, 22 samples were normal and 12 samples in the KRAS gene had a nucleoside change.Of the 12 samples, 11 nucleotide changes in codon 12 and 1 change in codon 13 of the gene were observed.There was no significant relationship between the KRAS mutationswith age, sex, tumor location, site of metastasis, king of pathology, grade, vital statues and lymph node, margin, vascular involvement (Table 2).

The 2-year overall survival (OS) for patients with KRAS wild-type vs. KRAS mutations has been shown in (Figure 1). The OS rate of the patients with KRAS wild-type was 73% and for KRAS mutations was 63%. There was no significant correlation between the two groups (P>0.05).

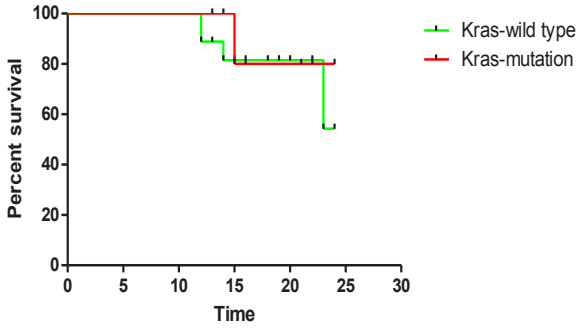

Figure 1. Two Year Overall Survival

Table 1. Study Characteristics by KRAS-Mutation Status (n=33)

	KRAS wild-type (N= 22) (%)	KRAS-mutated (N= 11) (%)	P-Value*
Age			0.66
<50	10(45)	6(55)	
50-59	6(27)	1(9)	
60-69	5(23)	3(27)	
70-79	1(5)	1(9)	
Sex			
Male	18(82)	8(73)	0.66
Female	4(18)	3(27)	
Tumor Location			0.31
Rectum	11(50)	4(36)	
Rectoigmoid	7(32)	4(36)	
Sigmoid	4(18)	1(9)	
Transverse	0(0)	1(9)	
Aescending	0(0)	1(9)	
Site of Metastasis			0.11
Liver	16(76)	5(42)	
Lung	3(14)	5(42)	
Liver + Lung	1(5)	1(8)	
Peritoneum	1(5)	0(0)	
Pelvis	0(0)	1(8)	
Kind of Pathology			1
Invasive Adenocarcinoma	21(95)	11(100)	
Mucinous Adenocarcinoma	1(5)	0(0)	
Grade			
Well	13(59)	9(82)	0.39
Mod	6(27)	1(9)	
Poor	2(14)	1(9)	

*P-value for X^2

Table 2. Nucleotide Changes in Codon 12 or 13 KRAS Gene

Number	Age	Sex	Site of Primary	Codon 12	Amenoacid Exchange	Codon 13	Amenoacid Exchange
1	22	Male	Rectum	GGT → GTT	GLY→ VAL		
2	34	Male	Rectosigmoid	GGT → GAT	GLY → ASP		
3	35	Female	Rectum				
4	37	Male	Rectosigmoid				
5	37	Male	Rectosigmoid	GGT → GTT	GLY → VAL		
6	38	Male	Rectosigmoid	GGT → AGT	GLY → SER		
7	38	Male	Rectosigmoid				
8	40	Male	Rectum	GGT → GAT	GLY → ASP		
9	43	Male	Rectum				
10	45	Male	Sigmoid				
11	45	Female	Sigmoid	GGT → UGC	GLY → CYS		
12	45	Male	Rectosigmoid				
13	45	Male	Rectosigmoid				
14	47	Female	Rectum				
15	49	Male	Sigmoid				
16	49	Male	Rectosigmoid				
17	51	Male	Rectosigmoid				
18	52	Male	Rectum				
19	57	Male	Rectum				
20	57	Female	Rectum				
21	58	Male	Rectum	GGT → GTT	GLY → VAL		
22	59	Male	Rectosigmoid				
23	59	Male	Rectum				
24	60	Male	Sigmoid				
25	62	Female	Rectum			GGC → GAC	GLY → ASP
26	62	Male	Rectum				
27	64	Male	Rectum				
28	64	Male	Rectum				
29	65	Male	Transverse	GGT → GAT	GLY → ASP		
30	65	Female	Ascending	GGT → GAT	GLY → ASP		
31	68	Female	Rectum				
32	71	Male	Rectosigmoid	GGT → GAT	GLY → ASP		
33	76	Male	Sigmoid				

Discussion

Mutation in KRAS gene is one of the most common oncogenic changes in various types of human cancer (Ansari et al., 2007).Emergence of KRAS mutated status is an alarming situation for CRC patients being treated with anti-EGFRs. Presence of KRAS mutations in a tumor treated with monoclonal antibodies is a sign of becoming refractory to treatments (Sameen et al ., 2016). A study recorded 12/26 (53.8%) deaths, 3/7 (57.1%) of patients with mutations and 9/19 (52.6%) of patients with wild type (Dobre et al., 2015).Mutation in codons 12, 13 KRAS is common in CRC. This produces an active KRAS protein resulting in the activation of the MAPK (mitogen activated protein kinase) cascade independently of EGFR activation (Naghibalhossaini et al., 2011) .

The median OS for patients with KRAS mutation at codon 12 was 27.3 months (Chang et al., 2014).Roughly, 90% of the activating mutations that are influential solitary amino acid replacement in the GTPase pocket and guide a block of the activity of KRAS-p21 protein, are recognized in codons 12 (GGT) and 13 (GGC) (Faghani et al., 2012) that mutations in codon 12 accounted for 79.3% of the mutations, the rest of them being in codon 13(Dobre et al., 2015). In codon 12, the most regularly found kinds of mutations are Gly to Asp(Dobre et al., 2013;Faghani et al., 2012;Geramizadeh , 2015)in concordance with evidence from Chinese and Caucasian mCRC patients (Li et al ., 2010;Mao et al., 2012)and Gly to Val transitions (Faghani et al., 2012)and in two studies from south of Iran [Dobre et al., 2012]and other study [Geramizadeh , 2015] was Gly to Cys.In our study 10/33(30%) deaths, 6/22(27%) of cases with mutations and 4/11(37%) of patients with wild type. 18 monthswas mean OS for 90 percent (codon 12) of patients with KRAS mutation. Most of the mutations were assigned to Gly12Asp and Gly12Val, respectively.A number of studies conducted in Caucasian and Asian CRC populations found no association between the prevalence of KRAS mutations and various clinicopathological parameters, including the gender and age of patients as well as tumor location, histological type and differentiation (Nakanishi et al., 2013; Zlobec et al., 2010; Chaiyapan et al., 2013) also we did not any logical relationship between KRAS mutations with age, sex, tumor location, site of metastasis, king of pathology, grade, vital statues and lymph node, margin, vascular involvement.

Our study was relatively similar to other studies. KRAS statues can help to treat patients more efficientlyand increase the survival of patients withmetastatic CRC. Moreover,more Kurdish people due to economic problems are not able to do this valuable genetic test. In final, we

need the more careful research of KRAS oncogene at the molecular level in the young population.

References

Madani SH, Sadeghi E, Rezaee A, et al (2015). Survey of HER2-neu expression in colonic adenocarcinoma in the west of Iran. *Asian Pac J Cancer Prev*, **16**, 7671-4.

Carethers JM (2015). Biomarker-directed targeted therapy in colorectal cancer. *J Dig Cancer Rep*, **3**, 5-10.

Vecchione L, Jacobs B, Normanno N, et al (2011). EGFR-targeted therapy. *Exp Cell Res*, **317**, 2765-71.

Bando H, Yoshino T, Tsuchihara K, et al (2011). KRAS mutations detected by the amplification refractory mutation system-Scorpion assays strongly correlate with therapeutic effect of cetuximab. *Br J Cancer*, **105**, 403-6.

Van Cutsem E, Köhne CH, Hitre E, et al (2009). Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med*, **360**, 1408-17.

Dobre M, Dinu DE, Panaitescu E, et al (2015). KRAS gene mutations - prognostic factor in colorectal cancer? *Rom J Morphol Embryol*, **56**, 671-8.

Perkins G, Pilati C, Blons H, et al (2014). Beyond KRAS status and response to anti-EGFRtherapy in metastatic colorectal cancer. *Pharmacogenomics*, **15**, 1043-52.

Rosty C, Young JP, Walsh MD, et al (2013). Colorectal carcinomas with KRAS mutation are associated with distinctive morphological and molecular features. *Mod Pathol*, **26**, 825-34.

Yoon HH, Tougeron D, Shi Q, et al (2014). KRAS codon 12 and13 mutations in relation to disease-free survival in BRAF-wild-type stage III colon cancers from an adjuvant chemotherapy trial (N0147 alliance). Clin Cancer Res,20, 3033-43.

Sameen S, Barbuti R, Milazzo P, et al (2016). Mathematical modeling of drug resistance due to KRAS mutation in colorectal cancer. *J Theor Bio*, **389**, 263-73.

Chang YY, Lin JK, Lin TC, et al (2014). Impact of KRAS mutation on outcome of patients with metastatic colorectal cancer. *Hepatogastroenterol*, **61**, 1946-53.

Dobre M, Comănescu M, Arsene D, et al (2013). K-ras gene mutation status in colorectal cancer: comparative analysis of pyrosequencing and PCR-RFLP. *Rom J Morphol Embryol*, **54**, 567-74.

Dobre M, Dinu DE, Panaitescu E, et al (2015).KRAS gene mutations - prognostic factor in colorectal cancer? *Rom J Morphol Embryol*, **56**, 671-8.

Faghani M, FakhriehAsl S, Mansour-Ghanaei F, et al (2012). The Correlation between Microsatellite Instability and the Features of Sporadic Colorectal Cancer in the North Part of Iran. *Gastroenterol Res Pract*, **2012**, 756263.

Geramizadeh B (2015). Molecular Biomarkers of Colorectal Cancer: A Review of Published Articles From Iran. *Ann Colorectal Res*, **3**, 30100.

Ansari R, Amjadi H, NorozbeigiN, et al (2007).Survival analysisof colorectal cancer in patients underwent surgical operationin Shariati and Mehr Hospital-Tehran, in a retrospective study. *Govaresh*, **12**, 7-15.

Naghibalhossaini F, Hosseini HM, Mokarram P, et al (2011). High frequency of genes' promoter methylation, butlack of BRAF V600E Mutation among Iranian colorectal cancerpatients. *Pathol Oncol Res*, **17**, 819-25.

Li FH, Shen L, Li ZH, Luo HY, et al (2010).Impact of KRAS mutation and PTEN expression on cetuximab-treated colorectal cancer. *World J Gastroenterol*, **16**, 5881-88.

Mao C, Zhou J, Yang Z, et al (2012). KRAS, BRAF and PIK3CAmutations and the loss of PTEN expression in Chinese patients with colorectal cancer. *PLoS One*, **7**, 36653.

Nakanishi R, Harada J, Tuul M, et al (2013).Prognostic relevance of KRASandBRAFmutations in japanese patients with colorectal cancer. *Int J ClinOncol*, **18**, 1042-48.

Zlobec I, Bihl MP, Schwarb H, et al (2010). Clinicopathological and protein characterization of BRAF-and K-RAS-mutated colorectal cancer and implications for prognosis. *Intl J Cancer*, **127**, 367-80.

Chaiyapan W, Duangpakdee P, Boonpipattanapong T, et al (2013).Somatic mutations of K-ras and BRAF in Thai colorectal cancer and their prognostic value. *Asian Pac J Cancer Prev*, **14**, 329-32.