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Relationship between KRAS and NRAS factors with clinicopathologic findings in patients with metastatic colon cancer

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Abstract

Introduction: Colorectal cancer (CRC) is the third common cancer among human and the fourth common reason of mortalities caused by cancers around the world. During recent years, EGFR-related molecular pathways are known as an important therapeutic pathway. High frequency of mutations of RAS family such as KRAS and NRAS and their rapid incidence in colon cancer indicates their high potential as a biomarker for early detection.

Materials and Methods: In this cross sectional retrograde study, patients with colorectal cancer referring to Golestan Razi and Poursina Hospitals in Iran were evaluated during years 2009-2018. The rates of KRAS and NRAS factors were evaluated on paraffinized pathology samples of patients with metastatic colon cancer. Then, the correlation between mutation in these two factors with other clinicopathological findings of patients such as age, gender, tumor grade, location of primary lesion, time to progression (TTP), family history and presence or absence of lymphovascular invasion was investigated.

Results: There was no significant correlation observed between occurrence of NRAS and KRAS with age group, family history and gender in the present study. But there was a significant statistical correlation between the rate of NRAS gene incidence with location of primary lesion and tumor grade. Finally, there was found a significant correlation between both KRAS and NRAS genes with TTP, so that TTP of patients reported less than patients without mutations in both groups.

Conclusion: The present study showed that presence of both mutations in KRAS and NRAS makes the prognosis of disease worth such a way the location of primary lesion and tumor grade are two effective factors in incidence of NRAS gene and lymphovascular invasion is the effective factor on KRAS gene incidence. also, TTP is lower among patients with mutations in both KRAS and NRAS genes.

Keywords: Colorectal cancer, KRAS, NRAS, Mutation

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Introduction

Colorectal cancer (CRC) is a neoplastic complex and multifactorial disease that as a result of a series of genetic changes, chromosomal abnormalities , mutations ,genetic and epigenetic changes and it has the ability to convert the natural epithelium of the colon to adenocarcinoma (1, 2). CRC is the third most common cancer in the world ,accounting for more than 10% of all new cancers worldwide (3). Approximately, millions of the new colorectal cancer identify around the world every year and nearly half a million people die due to this disease. The risk of colorectal cancer has different changes in the world, as according to studies carried out in the worldwide, the range has been variable between 13 to 66 percent and by comparing to developing areas, it is estimated that the chance of getting infected is more in developed areas (4, 5).

Changes in the cellular genome are affected by the expression or function of genes controlling cell growth and differentiation. Molecular study of cancer helps gene identification that varies in different types of tumors and it helps to explain the role of these genes in carcinogenesis. One of the different genetically changes is receiving the factor of epidermal growth factor receptor (EGFR) that is important in the molecule's target for treating colorectal cancer(6, 7). KRAS (Kirsten rat sarcoma) and NRAS (Neuroblastoma RAS) are the most important downstream molecules of the epidermal growth factor receptor signaling pathway.Three RAS human genes HRAS, KRAS and NRAS are known to be small GTP-GDP binding proteins and act as functional switches by coupling growth receptors to intracellular signaling pathways (6, 8, 9). KRAS and NRAS proto-oncogene encodes a binding protein to a guanosine tri phosphate /guanosine di- phosphate that it is located in intracellular membrane and plays important role in mitogenic transmission signal and cellular response adjustment to the extracellular stimulus such as growth factors ,cytokines and hormones (10, 11).

Researches has shown that mutations in KRAS and NRAS causing loss of GTPase activity from 30 to 50 percent of patients with colorectal cancer (12). High frequency of this mutation and its rapid onset of colon cancer showed the its high potential as a biomarker for the early detection (13). Currently, anti-EGFR antibody has been shown to be an effective therapeutic factor in CRC patients. However, patients with mutations in KRAS and NRAS do not show an appropriate therapeutic response to EGFR antibody. Hence, from the KRAS and NRAS status used as biomarkers to identify patients with mutations (14).

In this study due to increased prevalence CRC and also the impact of the role of gene mutations, especially mutations in RAS genes in the onset of the disease, we decided to investigate the relationship between the KRAS and NRAS factors with the findings of clinicopathologic in patients with the metastatic colon cancer.

Materials and Methods

In this cross-sectional study retrospectively, patients with colorectal cancer referred to Razi and Poursina hospital Gilan, Iran, during 2009 to 2018 were studied. Inclusion criteria includes all patients with metastatic colon cancer which was confirmed by CT scan or enhanced Carcinoembryonic antigen (CEA).Exclusion criteria also was the lack of willingness of the patient to follow the treatment. Before reviewing patients' cases, all stages of the study were explained and a consent letter were taken from all subjects. In this study, no money was received from patients and the laboratory foundation was responsible for the costs. Accordingly, 67 patients with colorectal cancer were included in the study and the amount of KRAS and NRAS factors were evaluated on a paraffin embedded pathologic specimens of patients with metastatic colon cancer under the supervision of an oncologist by PCR.

The samples from the primary location of the lesion, in case of metastases, was provided from the metastasis location again. Patients in relation to each gene were divided in two categories: 1) Wild type KRAS (no mutation) and none-wild type KRAS (Contains mutation), 2) Wild type NRAS (no mutation) and none-wild type NRAS (Contains mutation). The relationship between the mutations in these two factors with other findings to clinicopathologic patients include age ,sex , grade of tumor ,the lesion of primary ,TTP (Time to progression), family history and existence or non - existence Lymph vascular invasion was analyzed.

Statistical analyses

In this study, the collected data were coded and entered into SPSS 22 software. By the use of descriptive statistic, for the qualitative variable (frequency or percentage), normal mean quantitative variable, standard deviation, abnormal median and quadratic range was reported. In inferential experiments, for normal mean quantitative variable T -independent, otherwise nonparametric equivalent Man Whitney were used and for quantitative variable chi square or fisher exact test carried out. Significant level test was $p<0.05$.

Results

In this study, 67 patients with metastatic colon cancer were investigated. From these 67 samples, in terms of both KRAS and NRAS, 16 cases (23.9%) were not wild-type. Also 10 cases in terms of both KRAS and NRAS were not wild-type either. The average age of individuals in the study were 58.67±10.17 with range of 31 to 78 years of age. According to table 1, a higher percentage of patients (61.2%) were male and also in terms of the location of the primary lesion, more than half of the people (55.2%) rectosigmoid were reported. As results of this study showed that in terms of the tumor grade, more than half of the samples tested (56.7%) high grade were reported. Also, the average of TTP in patients were 10.13±4.58 months, 10 months median, 13 – 7 =IQR and the range of TTP was 2 to 25 months. According to the results, in the terms of family history, 26 of the 67 cases examined (38.8%) were positive and a higher percentage of cases (61.2%) were negative. In terms of lymph vascular invasion also evaluated in patients, 36 cases (53.7%) Lymph vascular invasion was found.

Table 1. Demographic and tumor characteristics in patients.

Variable		N	%
Age	30-60	33	49.3
	>60	34	50.7
Sex	Male	41	61.2
	Female	26	38.8
Primary lesion location	Ascending colon	7	10.45
	Descending colon	14	20.9
	Transverse colon	9	13.43
	Rectosigmoid	37	55.22
GRID Tumor	low	13	19.4
	intermediate	16	23.88
	high	38	56.72
TTP	Less than 10 months	40	59.7
	More than 10 months	27	40.3

In this study, the relationship between the gene expression of NRAS and KRAS with age group, gender, lymph vascular invasion, tumor grade, family history, the location of primary lesion and TTP were analyzed. On the basis of the relationship between gene expression of NRAS and KRAS with age group using Chi -square, no significant correlation was found and it is found that more than half of the patients by age distribution , NRAS and KRAS genes were reported as wild-type (Table 2). There was no significant relationship between NRAS and KRAS gene expression and sex with chi-square test and overall, more than half of the patients (male and female) were wild-type in terms of NRAS and KRAS genes (Table 2).

Table 2. Investigation of the relationship between KRAS and NRAS gene expression with demographic.

Variable			NRAS			KRAS		
			None wild	Wild	Total	None wild	Wild	Total
Age	30-60	N	11	22	33	7	26	33
		%	33.3	66.7	100	21.2	78.8	100
	>60	N	5	29	34	9	25	34

		%	14.7	85.3	100	26.5	73.5	100
	total	N	16	51	67	16	51	67
		%	23.9	76.1	100	23.9	76.1	100
	p-value*			0.074			0.614	
Sex	Male	N	13	28	41	12	29	41
		%	31.7	68.3	100	29.26	70.74	100
	Female	N	3	23	26	4	22	26
		%	11.5	88.5	100	15.4	84.6	100
	Total	N	16	51	67	16	51	67
		%	23.9	76.1	100	23.9	76.1	100
	p-value*			0.059			0.194	

The correlation between KRAS gene expression and the primary lesion location with Fisher test was not significant. However, the association between NRAS gene expression and the primary lesion location was significant ($p<0.05$). So that, in 57.1% of patients with primary lesion location in the descending colon, NRAS was mutated and reported as non-wild-type (Table 3). Also according to Table 3, no significant relationship was found between NRAS and KRAS gene expression with family history using chi-square test. The incidence of KRAS gene with a family history was similar to the NRAS results.

Table 3. The relationship between KRAS and NRAS gene expression with Primary lesion location and Family history.

			NRAS			KRAS		
			None wild	**Wild**	**Total**	**None wild**	**Wild**	**Total**
Primary lesion location	Ascending colon	N	3	4	7	3	4	7
		%	42.9	57.1	100	42.9	57.1	100
	Descending colon	N	8	6	14	5	9	14
		%	57.1	42.9	100	35.7	64.3	100
	Transverse colon	N	3	6	9	3	6	9
		%	33.3	66.7	100	33.3	66.7	100
	Rectosigmoid	N	2	35	37	5	32	37
		%	5.4	94.6	100	13.5	86.5	100
	total	N	16	51	67	16	51	67
		%	23.9	76.1	100	23.9	76.1	100
	p-value			0.059			0.113	
Family history	Positive	N	7	34	41	7	34	41
		%	17.1	82.9	100	17.1	82.9	100
	Negative	N	9	17	26	9	17	26
		%	34.6	65.4	100	34.6	65.4	100
	Total	N	16	51	67	16	51	67
		%	23.9	76.1	100	23.9	76.1	100
	p-value			0.101			0.101	

In the relationship between the gene expression of NRAS and Lymph vascular invasion using chi-square test, a significant relationship did not observe (p>0.05). According to Table 4, from 51 patients that in the terms of genes expression of NRAS were wild-type, 24 cases (47.1%) without Lymph vascular invasion and 27 (52.9%) had lymphovascular invasion. While there was a significant relationship between KRAS gene expression and lymphovascular invasion using chi-square test (p< 0.05).So that the highest rate of lymphovascular invasion in patients with mutations were observed in the gene KRAS.

In the study of gene expression NRAS and tumor grade, a significant relationship was found by using Fisher's test (p<0.05) and more than half of the patients who in terms of genes NRAS had mutations, in terms of tumor grade were in high stage. However, there was no significant relationship between KRAS gene expression and tumor grade using Fisher test (Table 4).

Table 4. The relationship between the expression of KRAS and NRAS genes with Lymphovascular invasion and tumor grade.

Variable			Lymphovascular invasion			P	Tumor Grade				P
			pos	neg	Total		high	intermediate	low	Total	
NRAS	Wild	N	27	24	51	0.817	24	15	12	51	0.022
		%	52.9	47.1	100		47.1	29.4	23.5	100	
	None wild	N	9	7	16		14	1	1	16	
		%	56.2	43.8	100		87.5	6.3	6.3	100	
	Total	N	36	31	67		38	16	13	67	
		%	53.7	46.3	100		56.7	23.9	19.4	100	
KRAS	Wild	N	23	28	51	0.011	26	14	11	51	0.264
		%	45.1	54.9	100		51	27.4	21.6	100	
	None wild	N	13	3	16		12	2	2	16	
		%	81.2	18.8	100		75	12.5	12.5	100	
	Total	N	36	31	67		38	16	13	67	
		%	53.7	46.3	100		56.7	23.9	19.4	100	

In comparing the TTP with the amount of NRAS and KRAS mutations, the results showed significant relationship of the TTP variables in both groups (p<0.05). Mean and standard deviation in wild and non-wild groups, in terms of NRAS genes were reported 10.8±4.7 and 8±3.2, while theses values for the gene KRAS were reported 10.76±4.3 and 8.13±5.0 respectively (Table 5).

Table 5. Investigation of the relationship between expression of KRAS and NRAS genes with TTP.

Variable		TTP					
		N	Maximum	Minimum	Median	Mean ± Std	P-value
NRAS	Wild	51	25	2	10	10.8±4.767	0.021
	None-wild	16	14	3	8	8±3.204	
	Total	67	25	2	10	10.13±4.582	
KRAS	Wild	51	25	2	10	10.76±4.302	0.009
	None-wild	16	24	3	7.5	8.13±5.005	
	Total	67	25	2	10	10.13±4.582	

Discussion

KRAS and NRAS mutations are important in the carcinogenesis of CRC and play a certain role in the efficacy of anti-EGFR therapy (15, 16). Among the RAS family, mutations in KRAS account for about 85% and NRAS for about 15% of all RAS mutations in human tumors (17). NRAS is identical to KRAS in the first 85 amino acids. However, unlike KRAS, NRAS is not activated by specific cytokines or growth factors (18).

In this study evaluation of 67 patients infected with CRC showed that in terms of both KRAS and NRAS, 23.9% of patients, were not wild-type. The average age of patients was 58.67± 10.17 and in terms of gender distribution , 61.2% of the individual were men.

In the study of Palomba and et al that were performed on 1284 patients, the mean age of people was reported 64 years, and about 60% of them were male. Mutation in KRAS gene was 35.6% and in NRAS was 4.1% of patients that it was different from the values obtained in our study.Also, 35.1% of individuals were under 60 years old and 64.9% were over 60 (19). In the study of Kadowaki et al that were performed on 813 patients, mutation in KRAS gene were 38% of patients. Mean age of individuals in the mutation group for KRAS were reported 64.7 and in those without mutation were reported 63.5. In their study, 53% of the mutations were male and 47% were female (20). Also in the study Velho et al (21), 35.3% of patients had mutations in KRAS oncogene, Brink et al (2) also collectively reported the frequency of mutations in KRAS oncogene that took on 737 samples of patients with colorectal cancer (diagnosis age between 57 and 76 years old), 37% were reported. In the study of Kawazoe et al that carried out between 2013 to 2014 on 264 patients with metastatic colorectal cancer, mutations in KRAS ,NRAS, BRAF and PIK3CA genes were examined. KRAS gene mutation in 37.9% and NRAS gene mutation in 2.4% of patients were found. 64% of patients with a mutation in the RAS gene were men (22) .

In the current stud, in terms of the location of the primary lesion, in more than half of the samples (55.2%) rectosigmoid were reported that was almost similar to Kavazai study (54.3). While in the study of Palomba, the highest rate of sample (40 %) were related to the ascending colon. But in their study, the incidence of RAS mutations was not significantly different in each colon(19). In the study of Isnaldi etal also had involved in descending colon (69%) (23).

In the investigation of the relationship between genes expression in the NRAS and KRAS with age group, gender, lymphovascular invasion, tumor grade, family history, the location of primary lesion and TTP, no significant relationship were observed between incidence rate of NRAS and KRAS with age, family records and gender. But between gene expression rates of NRAS, there was a significant statistical relationship with the location of primary lesion. As in 57.1% of patients with the location of primary lesion in the descending colon, NRAS contained mutations and were reported not wild-type. Also, about expression of KRAS gene, significant relationship was found with lymph vascular invasion and tumor grade. The amount of lymphovascular invasion in patients with mutant KRAS and wild-type KRAS was 81.3% and 45.1% respectively and the highest rate of lymphovascular invasion has been observed in patients with mutations in the KRAS gene. According to our results, 87.5% of patients with the NRAS gene had mutations and in terms of tumors grade, they were at high stage. It should be noted that there was a significant relationship between the expression of both KRAS and NRAS genes with TTP.

In the study of Palomba et al (19) on 1284 patients with metastatic colon cancer, there was no statistically significant relationship between the mutation in the KRAS and NRAS genes and age, sex, location of the primary lesion, tumor grade variables. Whilst no significant relationship was found between the amount of mutations in NRAS and KRAS with overall survival and Time to progression. Along with our study, Zhang et al found that there is no significant relationship between mutations RAS and family history (24). About the relationship between lymphovascular invasion with gene mutation RAS, Sayagués et al stated that there was a significant relationship between KRAS mutations and right side colon tumor location and absence of lymph vascular invasion (25). ChangM.D et al also in their study of RAS family gene in CRC cancer stated that between three KRAS ,NRAS and HRAS genes, only mutation in KRAS gene with

lymphovascular invasion have significant relationship (26).

In parallel to our results, it was shown that in the Kawadawaki et al study that carried on 813 patients with colon cancer in Japan, it was reported that over 65 years of age ,male gender, and the existence of mutations in KRAS gene causes the worst prognosis and less survival. So that in the mutated state compared to no mutation, 5 years DFS (Disease free survival) were reported 71% versus 77% respectively and 5 years OS (overall survival) were reported 80% versus 84% respectively. But between KRAS genes and variables such as age and tumor stage, lymph node metastasis no statistically significant relationship was reported(20). Also in the study of Palomba et al examined the linked mutations in KRAS, NRAS, BRAF, and PIK3CA genes with sex, age at diagnosis, anatomical location of primary CRC, tumor grading variables, and reported that there was no significant relationship between the mentioned variables and the mutation in these 4 genes(19). In the study of Kawazoe that mutation in KRAS, NRAS, BRAF and PIK3CA were analyzed, although the mutation in the RAS genes in male sex, location of primary rectum tumor and well differentiation degree was more but no significant statistical relationship found. In their study was reported that mutations in any of the genes mentioned in PFS (Progression free survival) mutation group have less period (1.2-9.5 months) compared to those without the mutation (4.6-8.7months). While the OS (Overall Survival) disease in each of mutation group has less period than those without mutations (3.6-8.6 months vs 1.34-1.3 months) (22).

In the study of Jouini et al also the relationship between mutations in the RAS genes and variables such as age, gender ,age groups and perineural invasion, metastasis, vascular emboli and degree of differentiation was analyzed. In their study, mutations in the RAS genes have significant relationship with the degree of differentiation. 82% well differentiation patients have mutation in the RAS gene (14). In a meta-analysis by Therkildsen et al that carried on in 2014, was reported that mutations of both KRAS and NRAS genes with overall survival (OS) and progression-free survival (PFS) have less common disease and the existence of these mutations make them resistant to anti-EGFR drugs (27). Rebersek et al also stated in a study that median time to recurrence of the disease in patients after primary treatment of operable disease with a non-mutated *KRAS* gene was shorter than in patients with mutated *KRAS* gene (20 *vs*. 21 months), but the difference was not significant(28). In another study, Gasparini et al. express that the patients with wild-type tumors had a statistically significant better TTP as compared to those with *RAS* mutated disease with 7.4 ± .85 weeks versus 5.2 ± .91 weeks (29).

In general, there appears to be a mutation in the KRAS and NRAS gene can be used as a suitable biomarker for assessing response to targeted treatment. One of the limitations of this study was the expensive cost of the test and lack of samples despite of the analyze in a ten-year study due to the death and errors in patient records. It is thought that an increase in the sample size might affect the results and if the study carried on broad samples, better results can be achieved.

Conclusions

Our study showed that the incidence rate of NRAS and KRAS have no relationship with age group, family history and gender, but patients whose primary lesion location was located in descending colon, the NRAS gene had a mutation. In addition, in more than half of the patients with NRAS mutations, the tumor grade was high. Also, the rate of lymphovascular invasion in patients without KRAS mutation, much less patients with mutation were reported. TTP was also lower in both KRAS and NRAS genes in patients with mutations than in non-mutated patients.

Author contribution

HSS and **MMA** supervised and managed the project and also edited and revised the manuscript. **FN** and **KM** collected the data and wrote the primary draft of the manuscript.

Conflict of interest

No potential conflict of interest was reported by the authors.

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