Research Article

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KRAS and NRAS Mutations in Moroccan Patients with Colorectal Cancer

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Abstract

Objective: Activating mutations in the RAS proto-oncogene (KRAS, HRAS and NRAS) play a crucial role in carcinogenesis and drug resistance in many cancers including, colorectal cancer. The aim of the present study was to evaluate the frequencies of KRAS and NRAS oncogenic mutations in Moroccan CRC patients.

Patients and methods: In the present study, formalin-fixed paraffin-embedded CRC specimens from 114 Moroccan CRC patients were analyzed. To detect mutations in codons 12, 13 and 61 of the KRAS and NRAS genes, the pyrosequencing technique was used.

Results: 114 colorectal cancer patients were included in this study, 64 were males and 50 were females, the primary tumor was the most frequent type (78.1%) and the commonest histological type was ADK (77.2%). KRAS mutation was found in 49 of all CRC patients and the most frequent KRAS mutations were 35G>T, 35G>A and 38G>A. On the other hand NRAS mutation was detected only in 6 patients. No statistically significant relationship between CRC patient's characteristics and RAS mutations was found.

Conclusion: Our results suggest that the KRAS mutation is more frequent among Moroccan patients with CRC, which is in agreement with the most previous studies. Further studies, with a larger number of CRC patients, are needed to confirm our findings.

Keywords: KRAS, NRAS, Colorectal cancer, Morocco

Introduction

Colorectal Cancer (CRC) is defined by the malignant proliferation of cells lining the walls of the colon or rectum. CRC is the third most common cancer in the world after lung and breast cancer. Each year, over one million people are diagnosed with colorectal cancer and over half a million die from it [1-5]. The incidence of this cancer varies from country to country due to differences in diet, lifestyle, and genetic background between populations [6]. This cancer most often occurs sporadically and the hereditary form represents about 5% [7]. The

adenocarcinoma is the most common histological type [8]. The risk factors for CRC including, cholecystectomy, inflammatory bowel disease, irradiation, obesity, smoking, high alcohol consumption, diet rich on red or processed meats [9].

Activating mutations in the RAS proto-oncogene family (KRAS, HRAS and NRAS) played an important role in carcinogenesis, progression and drug resistance in many cancers by altering biological functions such as, apoptosis, cell proliferation and differentiation [10]. The frequencies of these mutations vary according

to ethnic groups and geographic factors.

In CRC, the KRAS gene is frequently mutated mainly in the codons 12, 13, 59, 61, and 146. Mutations in this gene represent about 27 to 56% of CRC cases and have been reported to be a negative prognostic marker that induces resistance against to treatment with anti-EGFR antibodies cetuximab and panitumumab [10-13]. The NRAS gene is rarely mutated in this cancer in about 1 to 7% of cases [10-12].

The aim of the present study was to estimate the frequencies of KRAS and NRAS mutations using pyrosequencing in a sample of a Moroccan population.

Patients and Methods

114 formalin-fixed paraffin-embedded CRC specimens were analyzed in the present study. Clinical and demographic factors, such as: age, sex, Tumor type, Histological type, etc were collected from patient records. The study was approved by the local ethics committee of the Hassan II University and written informed consent was obtained from each patient Table 1.

Table 1: Clinical Characteristics of the 114 colorectal cancer patients.

	Cases (N=114)
Sex N (%)	
Females	50 (43.9%)
Males	64 (56.1%)
M:F ratio	1.28
Age years (Mean)	55.41
Range	19-84
≤50	44(38.6%)
51-64	50 (43.9%)
≥65	20 (17.5%)
Tumor type	
Primary	89 (78.1%)
Metastasis	23 (20.2%)
local recurrence	2 (1.8%)
Tumor percentage	
< 50	70 (61.4%)
≥50	44(38.6%)
Histological type	
ADK	88 (77.2%)
Others	26 (22.8%)

Genomic DNA extraction

The genomic DNA was purified from formalin-fixed paraffin-embedded tissues samples using the QIAamp DNA FFPE Tissue Kit (Qiagen, Valencia, CA, USA), extracted DNA samples were quantified on the NanoDrop. Carry out the DNA extraction according to the instructions in the kit handbooks. For detecting KRAS and NRAS samples were analyzed by quantitative PCR and Pyrosequencing.

PCR amplification and Pyrosequencing

PCR was used for amplifications of a region containing codon 12, codon 13, and codon 61. PCR assay was performed in a reaction volume of 20 µL using 12.5 µL PyroMark PCR Master Mix, 2.5 µL CoralLoad Concentrate, 10x, 1 µL PCR Primer and 4 µL Water (H₂O, supplied). The PCR conditions were as follows: Initial denaturation at 95°C for 15min, followed by 40 cycles of 95°C for 20 sec, 53°C for 30s and extension at 72°C for 20 sec and then final extension at 72°C for 5min). Unmethylated control DNA was included in the product as a positive control for PCR and sequencing reactions. In addition, a negative control (without template DNA) was included. After PCR, the amplicons are immobilized on Streptavidin Sepharose High Performance beads. Single-stranded DNA is prepared and the corresponding sequencing primers anneal to the DNA. The KRAS, NRAS (codons 12, 13, 61) oncogenes mutations were detected by the pyrosequencing technique using Pyro kit (Qiagen).

Statistical analysis

Statistical analyses were performed using the SPSS software package version 17.0 (SPSS Inc., Chicago, IL, USA). For all the analyses, *P* values <0.05 were considered statistically significant.

Results

114 colorectal cancer patients were included in the present study, 64 (56.1%) were males and 50 (43.9%) were females; with a sex ratio of 1.28. The mean age of our patients was 55.41 years; the majority of our patients were aged less than 50 years (61.4%). For the tumor type, the primary tumor was the most dominant type with a frequency of 78.1%. The most frequently histological type ADK, was seen in 77.2% of all cases. The tumor percentage was less than 50 at diagnosis in 61.4% of patients. The characteristics of the 114 CRC patients are summarized in table 1.

For the RAS mutations, a KRAS mutation was found in 49 of CRC patients (43 %). The most frequent mutations

were 35G>T (36.7%), 35G>A (14.3%) and 38G>A (14.3%). KRAS exon 2 mutations were detected in 46 patients (93.9%) and mutations outside KRAS exon 2 were found in 6% of our patients. On the other hand NRAS mutation was detected in 6 patients (5.3%) and the most common mutations were 181 C>A (2.6%) and 182 A>G (1.8%) (Table 2). We did not find any significant correlation between patients characteristics age, sex, Tumor type, etc and RAS mutations (Table 3).

Table 2: KRAS and NRAS mutations among the 114 colorectal cancer patients.

		Cases (N=114)
KRAS N (%)		
	Wild type	58 (50.9)
	Mutated	49 (43)
NRAS N (%)		
	Wild type	101 (88.6)
	Mutated	6(5.3)
KRAS(% of mutated cases)		
exon 2	38G>A	7(14.3%)
Codons 12, 13	35G>T	18 (36.7%)
	35 G>A	7 (14.3%)
	34G>T	4 (8.2%)
	37G>T	1 (2.0%)
	34G>A	4(8.2%)
	35G>C	3 (6.1%)
	34G>C	1 (2.0%)
exon 3	182A>T	1(2.0%)
Codons 61	183A>G	1 (2.0%)
	175G>A	1 (2.0%)
exon 2	436G>A	1(2.0%)
NRAS(% of mutated cases)		
exon 3	181 C>A	3 (50%)
Codons 61	182 A>G	2 (33.3%)
	181 A>G	1 (16.7%)

Discussion

In our study CRC was more common among males, a similar result was obtained by other authors [14-18]; this increased susceptibility could be explained by genetic and physiological differences between men and women, men are more likely to use alcohol and tobacco and more likely to consume red meat.

The mean age of our patients was 55.41 and the most frequent age range was 51 to 64 years, these results were in agreement with Greater Casablanca cancer registry report 2012 and with Jouini et al findings in the J Cancer Biol Therap, 5(1): 254-259 (2019)

Tunisian population [19,20]. The most tissue specimens were from primary tumors, a similar result was reported by Jouini, et al. and Lee et al. [20,21].The majority of our patients were diagnosed with adenocarcinoma, this result was similar to that reported by Lee et al and Rindi et al. [8,21].

Table 3: Association of clinical Characteristics and RAS mutations in colorectal cancer patients.

NRAS			KRAS			
	N	P	P value	N	P	P value
Sex N						
Females	44	3	0.75	25	22	0.85
Males	57	3		33	27	
Age years						
≤50	39	2	0.63	24	17	0.6
51-64	44	2		25	21	
≥50	18	2		9	11	
Tumor type						
Primary	80	5	0.93	43	42	0.29
Metastasis	19	1		14	6	
Local recurrence	2	0		1	1	
Tumor percentage						
< 50	65	3	0.47	37	31	0.95
≥50	36	3		21	18	
Histological type						
ADK	79	36	0.24	48	36	0.24
Others	22	13		10	13	

We did not find any correlation between the clinical parameters such as age, sex, stage of disease with RAS mutations, our results agreed with the observations of Most of the previous studies [21-25].

The current study results showed that the KRAS gene mutation was seen in 43% of all CRC patients. Similarly, it was reported in the literature that the frequency of this activating mutation accounts for approximately 24%-43% of all CRC patients [20,22,26,27].

NRAS mutations were observed in 5.3% of the 114 patients, which agreed with the reported frequencies (3-20%) in the literature [10-12,20,28-30]. The KRAS exon 2 mutations were the most common mutations in our work, among these mutations, the most frequent were 35G>T (36.7%), 35G>A (14.3%) and 38G>A (14.3%) this result was comparable with those obtained in other studies [20,31,32]. Similar to our results, a low incidence

of outside mutations KRAS exon 2 has been detected in CRC cancer with frequencies between 3% and 31% [20,28-30,33,34].

For the KRAS mutation, it has been reported that the exon2 mutations influence the anti-EGFR therapy efficacy; patients with KRAS exon 2 wild type genotype are sensitive to anti-EGFR therapy and mutations outside of KRAS can cause resistance to anti EGFR monoclonal antibodies, for these reasons, the analysis of KRAS mutations should be included in routine clinical practice to improve the clinical response and survival of CRC patients [35-37].

Conclusion

The present study suggests that the KRAS mutation is more frequent while the NRAS mutation is rare among Moroccan patients with CRC, which is in agreement with the most previous studies among different ethnic groups. Further studies with large population size are needed to confirm these results.

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