RESEARCH ARTICLE

Clinical Significance of Somatic Mutations in *RAS/RAF/MAPK* Signaling Pathway in Moroccan and North African Colorectal Cancer Patients

Soukaina Benmokhtar[1,2]*, Abdelilah Laraqui[1,3], Fatima El Boukhrissi[4], Farida Hilali[1], Tahar Bajjou[1], Meryem Jafari[1], Sara Elzaitouni[1], Walid Baba[1], Bouchra El Mchichi[3], Hicham Elannaz[3], Idriss Amine Lahlou[3], Hafsa Chahdi[5], Mohamed Oukabli[5], Tarik Mahfoud[6], Rachid Tanz[6], Mohamed Ichou[6], Khaled Ennibi[3,7], Nadia Dakka[8], Yassine Sekhsokh[1]

Abstract

Background: Mutations in *RAS* (*KRAS, NRAS*) and *BRAF* genes are the main biomarker predicting response to anti-EGFR monoclonal antibodies in targeted therapy in colorectal cancer (CRC). **Objective:** Our study aims to evaluate the frequencies of KRAS, NRAS and BRAF mutations and their possible associations with clinico-pathological features in CRC patients from Morocco. **Methods:** DNA was extracted from 80 FFPE samples using the QIAamp DNA FFPE-kit. *RAS* and BRAF mutations were assessed by pyrosequencing assays using Qiagen, KRAS Pyro®kit 24.V1, Ras-Extension Pyro®kit 24.V1 and BRAF Pyro®Kit 24.V1, respectively, and carried out in the PyroMark-Q24. **Results:** RAS mutations were identified in 57.5% (56.2% in KRAS, 8.8% in NRAS). In KRAS gene, exon 2 mutations accounted for 93.3% (68.9% in codon 12, 24.4% in codon 13). Within codon 12, G12D was the most prevalent mutation (37.7%), followed by G12C (13.4%), G12S (8.9%) and G12V (6.6%). Within codon 13, the most frequently observed mutation was G13D (22.3%). The mutation rates of exon 3 and 4 were 15.6% and 13.3%, respectively. In exon 3 codon 61, 2.3% patients were detected with two concurrent mutations (Q61R, Q61H), and 4.4% with three concurrent mutations (Q61R, Q61H, Q61L). In NRAS gene, the mutation rates of exon 2, 3 and 4 were 57.1%, 28.6%, and 14.3%, respectively. G13A and Q61H were the most common mutations, accounting for 42.9% and 28.5%, respectively. There were 13% patients with concurrent KRAS/NRAS mutation and 4.3% wt KRAS with NRAS mutations. No mutations were identified in BRAF gene. In both sexes, KRAS codon 12 mutations were associated with higher stage III/IV tumors. Moreover, Patients whose tumor is in the proximal colon (56.3%) are more likely to harbor KRAS mutations than those tumor located in rectum (25%). **Conclusion:** *RAS* mutations could be useful in future target anti-EGFR therapy and molecular CRC screening strategy in Morocco.

Keywords: *KRAS- NRAS- BRAF*- Colorectal cancer- Pyrosequencing

Introduction

Colorectal cancer (CRC) is the third most diagnosed cancer and is the fourth leading cause of death worldwide. There were 1.93 million estimated new diagnosed CRC cases and 0.94 million CRC caused deaths in 2020 worldwide (Xi et al., 2021). The CRC-global burden is expected to increase by 60% causing 2.2 million new cases and 1.1 million annual deaths by 2030 (Arnold et al., 2017). CRC incidence and mortality rates vary widely worldwide, with distinct gradients across human development levels and its trends point towards widening

[1]*Laboratory of Research and Biosafety P3, Mohammed V Military Teaching Hospital, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco.* [2]*Laboratory of Human Pathologies Biology and Genomic Center of Human Pathologies, Department of Biology, Faculty of Sciences, Mohammed V University, Rabat, Morocco.* [3]*Sequencing Unit, Laboratory of Virology, Center of Virology, Infectious and Tropical Diseases, Mohammed V Military Teaching Hospital, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco.* [4]*Sidi Mohamed Ben Abdellah University, Faculty of Medicine and Pharmacy of Fez, Morocco.* [5]*Department of Pathology, Mohammed V Military Teaching Hospital, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco.* [6]*Department of Medical Oncology, Mohammed V Military Teaching Hospital, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco.* [7]*Center of Virology, Infectious and Tropical Diseases, Mohammed V Military Teaching Hospital, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco.* [8]*Faculty ofMedicine and Pharmacy, Rabat, University Mohammed V, Morocco. *For Correspondence: soukainabenmokhtar@gmail.com*

disparities and an increasing burden in countries in transition (Arnold et al., 2017). CRC is now recognized as one of the clearest markers of the disease transition in societies undergoing socioeco¬nomic development and transition to a lifestyle more typical of industrialized countries (Fidler et al., 2017).

In Morocco, CRC ranks first among gastro-intestinal cancers and it is the third most commonly diagnosed cancer (Fondation Lalla Salma Pévention et traitement des cancers, 2016). According to the data on GLOBOCAN 2020, there were 4324 new CRC patients and 2374 deaths, accounting for 7.3% and 2.8% of all cancers, respectively (Ferlay et al., 2020). There was a clear rising trend in CRC incidence with age-standardized increases in incidence rates of 3.8 to 8.4 and from 2.6 to 7.4 per 100,000 in men and women, respectively. CRC patients are a young population and are diagnosed between 55 and 59 years for both sexes (Fondation Lalla Salma Pévention et traitement des cancers, 2012). The detection rates of advanced adenomas and CRC were 4.0 in 1000 and 0.5 in 1000 screened individuals, respectively (Selmouni et al., 2017).

CRC is a complex and genetically heterogeneous disease involved in oncogene and tumor suppressor genes, as well as genes involved in DNA damage recognition and repair. CRC includes also different categories of tumors based on their specific spectrum of mutation and molecular phenotype, driving various oncogenic signaling pathways (Miele et al., 2020). The RAS-MAPK pathway is one of the most deregulated and extensively characterized pathways in CRC, with KRAS being the most frequently mutated gene. RAS oncogenes (KRAS and NRAS) encode small membrane-bound GTPase proteins that regulate cellular proliferation, differentiation, and survival. Under physiologic conditions, RAS proteins cycle between their GTP-bound active and GDP-bound inactive states to regulate the activation of downstream effectors proto-oncogene serine/threonine kinase (RAF), MAPK kinase (MEK), and extracellular-signal-regulated kinase (Kano et al. 2016). The BRAF oncogene encodes a protein belonging to the raf/mil serine/threonine kinases family that plays a role in intracellular signaling and cell growth, and is a downstream effector of KRAS in the MAPK signaling pathway. Mutations in BRAF gene will lead to cancer development and progression (Karimi et al., 2021; Bashir et al., 2022).

The frequency rates of KRAS, NRAS, and BRAF mutations in CRC differ among populations. Our study aims to evaluate the frequencies of KRAS, NRAS and BRAF mutations and their possible associations with clinico-pathological features in CRC patients from Morocco Materials and Methods

Materials and Methods

Subjects study

Between September 2020 and December 2021, a total of 80 FFPE specimens obtained from newly diagnosed CRC patients were screened for clinically relevant mutations in RAS and BRAF genes at the Laboratory of Research and Biosafety P3, Mohammed V Military Teaching Hospital at Rabat, Morocco. All tissue samples

that were extracted from primary or metastatic tumor through surgical resection or endoscopic biopsy were FFPE, and histologically confirmed.

Clinicopathological data were recorded from the medical records, including: histologic subtype, histologic grade, tumor site, vascular invasion, and perineural invasion. Primary tumors located in the cecum and ascending colon were defined as proximal tumors, whereas tumors located in the splenic flexure, descending colon, and sigmoid colon were defined as distal tumors. Detailed information on demographic factors, anthropometric characteristics, and lifestyle habits were also collected. The exclusion criteria included the presence of any other cancers, and prior anti-cancer treatment. All participants were informed about the significance of molecular testing and signed an informed consent form prior to molecular genetic testing.

Genomic DNA extraction

DNA was extracted from five slices of 10 µm FFPE tissues using the QIAamp® DNA FFPE Tissue kit (QIAamp DNA FFPE tissue, Qiagen, GmbH, Hilden, Germany). DNA concentrations were assessed with the fluorometric method based on the binding of double-stranded DNA (dsDNA)-selective fluorescent dyes (dsDNA) (Qubit 4.0 Fluorometer/Life Technologies, Invitrogen). All steps were performed following providers' guidelines.

Genotyping of Full RAS and BRAF

KRAS, NRAS and BRAF gene mutations were assessed by pyrosequencing assays using Qiagen, K-Ras Pyro®kit 24.V1, Ras-Extension Pyro®kit 24.V1 and BRAF® Pyro® Kit 24.V1. The target sequence covering the polymorphic site was amplified with one of the specific biotinylated primers. Briefly, PCR was used for amplifications of a full RAS region containing codon 12, codon 13, and codon 61 in KRAS and NRAS, as well as codons 600 and 464-469 in BRAF gene. The PCR assay was performed in a reaction volume of 20 µl using 12.5 µl PyroMark PCR Master Mix, 2,5µl CoralLoad Concentrate, 10X, 1 µl PCR Primer and 4 µl Water (H_2O, 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 incorporated in the product as a positive control for PCR and sequencing reactions. A negative control (without template DNA) was included. We subsequently immobilized, washed, and denatured the amplified products using the vacuum workstation and subjected those products to pyrosequencing using the PyroMark Q24 system (Qiagen, PyroMark Q24 MDx V2.0), Germany).

The pyrosequencing results were analyzed using the PyroMark-Q24 version 2.0.6 software (Qiagen, PyroMark Q24 MDx (version 2.0), Germany), which identifies the presence of a specific mutation and its percentage. Manufacturer-supplied Limits of detection (LOD) thresholds were used to call a mutation for LOD studies (≥ % LOD is positive). Real-time curves and programs were interpreted according to the kit

instructions and PyroMark ID software (Qiagen) allowed determination of mutant allelic frequency according to relative peak height.

Statistical Analysis

The data were analyzed by chi-square or Fisher's exact tests to study the correlation between the clinicopathological features and the occurrence of a particular mutation. The statistical analysis used the software IBM SPSS test version 23, with p < 0.05 taken as the threshold for a significant difference.

Results

Clinico-pathological characteristics of FFPE specimens

The clinicopathological features of the 80 CRC patients are summarized in Table 1. The primary tumor was localized in the colon and rectum in 45 (56.3%) and 20 (25%) of CCR patients, respectively. Histological analysis has demonstrated that 25 (31.3%) of the patients are high-differentiated adenocarcinomas, 46 (57.5%) are moderate-differentiated adenocarcinomas and 9 (11.3%) are low-differentiated adeno-carcinomas. At diagnosis, 6 (7.5%), 46 (57.5%), 28 (35%) patients had stage II, III, and IV carcinomas, respectively. The most common metastatic location is observed as the liver (11/80, 13.8%).

The baseline demographic characteristics of CCR patients

There were 52 (65%) males and 28 (35%) females; with a gender ratio of ~2:1. The mean age at tumor collection was 62 ± 10.6 years (range: 29-85 years) and the majority of patients were aged less than 60 years (35%, 28/80). The median age at CRC diagnosis is younger for rectal cancer (63 ± 16 years) than for colon

cancer (69 ± 15 years). For colon cancer, the median age at the time of diagnosis for men was 62 ± 7.9 years and for women was 58.5 ± 14.5 years. For rectal cancer, it is 62 ± 7.3 years for both men and women. CRC peaked in the 29 - 85 years old age group. About 5% of patients were younger than 40 years, 20% were 70 years and older.

Status of full RAS and BRAF gene mutations

RAS (KRAS and NRAS) mutations were identified in 57.5% (46/80) with 56.2% (45/80) in KRAS and 8.8% (7/80) in NRAS. In KRAS gene, exon 2 mutations accounted for 93.3% patients including 68.9% (31/45) at codon 12 and 24.4% (11/46) at codon 13. Within codon 12, G12D was the most prevalent mutation (37.7%), followed by G12C (13.4%), G12S (8.9%) and G12V (6.6%). Within codon 13, the most frequently observed mutation was G13D (22.3%). Outside exon 2, the mutation rates of exon 3 and exon 4 were 15.6% and 13.3%, respectively. In exon 3 codon 61, 2.3% patients were detected with two concurrent mutations (Q61R, Q61H), and 4.4% with three concurrent mutations (Q61R, Q61H, Q61L). Outside exon 2, the mutation rates of exon 3 were 8.8% (4/45). In exon 4, the mutation rate was 4.4% (2/45) including 2.2% (1/45) in both codon 117 and codon 146. In KRAS codons, concurrent mutations were identified in 6.7% (3/45) including 2.3% (1/45) cases, with two distinct mutations (Q61R, Q61H) and 4.4% (2/45) with three distinct mutations (Q61R, Q61H, Q61L) in codon 61. In NRAS gene, the mutation rates of exon 2, exon 3 and exon 4 were 57.1% (4/7), 28.6% (2/7), and 14.3% (1/7), respectively. G13A and Q61H were the most common mutations and were found in 42.9% (3/7) and 28.5% (2/7) respectively. There were 13% (6/46) cases detected with both KRAS and NRAS mutations and 4.3% (2/46)

Table 1. Tumor Characteristics of 80 FFPE Specimens with Colorectal Cancer

Characteristics	No	%	KRAS			NRAS		
			Wild-type	Mutant	p-value	Wild-type	Mutant	p-value
Age (Years)								
Age<60	28	35	11	17	0.555	27	1	0.229
Age>60	52	65	24	28		46	6	
Gender								
Man	52	65	23	29	0.906	48	4	0.648
Women	28	35	12	16		25	3	
Primary tumor localization								
Rectum	20	25	14	6	0.022*	19	1	0.688
Colon	45	56.3	15	30		41	4	
Others	15	18.8	6	9		13	2	
Tumor histology (differentiated adenocarcinomas)								
High	25	31,3	11	14	0.793	22	3	0.712
Moderate	46	57.5	21	25		43	3	
Low	9	11.3	3	6		8	1	
Stages								
Stage II	6	7.5	3	3	0.162	6	0	0.701
Stage III	46	57.5	16	30		42	4	
Stage IV	28	35.0	16	12		25	3	

Table 2. Frequency and Distribution of KRAS and NRAS Mutations

Gene	Exon	Nucleotide Substitution	Codon Substitution	Amino Acid Substitution	Number	%
		c.35G>A	GGT>GAT	p.G12D	17	37.7
		c.34G>T	GGT>TGT	p.G12C	6	13.4
	2	c.34G>A	GGT>AGT	p.G12S	4	8.9
		c.38G>A	GGC>GAC	p.G13D	11	24.4
		c.35G>T	GGT>GTT	p.G12V	3	6.6
KRAS		c.35G>C	GGT>GCT	p.G12A	1	2.2
		c.182A>G	CAA>CGA	p.Q61R	1	2.2
	3	c.183A>C	CAA>CAC	p.Q61H	2	4.4
		c.182A>T	CAA>CTA	p.Q61L	1	2.2
	4	c.436G>A	GCA>ACA	p.A146T	1	2.2
		c.351A>C	AAA>AAC	p.K117N	1	2.2
	2	c.38G>C	GGT>GCT	p.G13A	3	42.9
		c.34G>C	GGT>CGT	p.G12R	1	14.2
NRAS	3	c.183A>T	CAA>CAT	p.Q61H	2	28.5
		c.175G>A	GCT>ACT	p.A59T	1	14.2
	4	c.351G>C	AAG>AAC	p.K117N	2	2.5
		c.436G>A	GCA>ACA	p.A146T	1	14.2

wt KRAS was identified with mutations in the NRAS gene. No mutations in the BRAF gene were found in our patients with CRC. The detailed distribution of the KRAS and NRAS identified mutations in 46 FFPE specimens have been given in Table 2.

Association between full RAS status and clinico-pathologic characteristics

Correlation of KRAS mutations with gender and age showed that KRAS mutations were slightly higher in

Table 3. Association between RAS Status and Clinicopathological Features

Characteristics	RAS wild-type	RAS Mutants	p-Value
Age (Years)			
Age< 60	11 (32.3)	17 (36.9)	0.674
Age>60	23 (67.6)	29 (63.04)	
Gender			
Female	12 (35.2)	15 (32.6)	0.805
Male	22 (64.7)	31 (67.3)	
Primary tumor localization			
Colon	15 (44.1)	30 (65.2)	0.223
Rectum	13 (38.2)	8 (17.3)	
Others	6 (17.6)	8 (17.3)	
Tumor Histology			
High	11 (32.3)	13 (28.2)	
Moderate	14 (41.1)	26(56.5)	0.656
Low	9 (26.4)	7(15.2)	
Stages			
II	2 (5.8)	3(6.5)	
III	19 (55.8)	31(67.3)	0.318
IV	13 (38.2)	12(26.0)	

women (57.1%) than men (55.7%), although the difference was not statistically significant. KRAS mutations were frequent in patients in the age range between 40 and 69 years and less frequent in patients younger than 40 years. The mean age of patients presenting KRAS mutations was 72 years for CCR patients with normal KRAS profiles. However, No significant differences were observed. KRAS codon 12 mutations, but not codon 13 mutations, were associated with higher tumor stages (III-IV) than those of wt carcinomas ($p < 0.05$). Patients whose tumor is in the proximal colon (56.3%) are more likely to harbor KRAS mutations than those tumor located in rectum (25%) in both sexes. NRAS mutations did not seem to be associated with any of the molecular features that were examined. Association between RAS status and clinicopathological features are summarized in Table 3.

Discussion

CRC exhibits KRAS mutation rates of ≈33% in the Catalogue of Somatic Mutations in Cancer (COSMIC) database comprising ≈75,000 tested samples. The frequencies of 40%–45% suggested by the other datasets are based on small sample sizes of fewer than 500. Notably however, the private Foundation Medicine dataset comprising 13,336 colorectal samples reports a KRAS mutation frequency of ≈50% (Serebriiskii et al., 2019). According to the COSMIC database, mutations at codon 12 (G12A, G12V, G12S, G12R, G12C, G12D) are the most prominent (>90%), followed by those affecting codon 13 (G13D and G13C), codon 61 (Q61L, Q61R, Q61H), codon 146 (A146T, A146V, A146P), and codon 117 (K117N). NRAS mutations are found in 5%-10% of CRC. NRAS is mutated in the same codon of KRAS (therefore, these positions are true hot-spot mutations), particularly in exon 2 (found in approximately 3%-5%

of CRC) and exon 3 (2%-6% of CRC). BRAF mutations are identified in about 8%-12% of CRC patients and 90% of all identified mutations are a T1799A transversion in exon 15, which results in a valine amino acid substitution (V600E) (Cantwell-Dorris et al., 2011).

In our study, RAS mutations were identified in 57.5% (56.2% in KRAS and 8.8% in NRAS) of all 80 patients with CRC, respectively. No BRAF gene alterations were found. The prevalence of KRAS and NRAS mutations in our cohort is higher than that reported by previous Moroccan studies which is within the 23.9% to 51% range for KRAS mutations (El Agy et al., 2021; Houssaini et al., 2020; Dehbi et al., 2019; Jadda et al., 2014; Marchoudi et al., 2013; Bennani et al., 2010;) and 2% to 5.3% range for NRAS mutations (Houssaini et al., 2020; Bennani et al., 2010), respectively. There are some explanations for this difference. First, we analyzed the RAS mutations using the Pyrosequencing technology, which is considered to show higher sensitivity (5%) than that of other screening methods. A study demonstrated that pyrosequencing detected 17.9% of the KRAS mutations in patients with KRAS wt using direct sequencing alone (Tougeron et al., 2013). Second, the previous Moroccan studies have examined the distribution of KRAS mutations from archived FFPE tissues. The Length of formalin fixation and FFPE specimens archiving process often causes cross linking and degradation-fragmentation of the DNA molecules. These alterations have negative consequences on the quality and quantity of extracted DNA from FFPE, and inevitably adversely affect downstream analyses, which in turn would impact the accuracy of genetic tests to detect mutated genes (Gao et al., 2020; Gill Ferreira et al., 2014).

In our study, the majority of the KRAS gene mutations were at exon 2 within 93.3%. Within codon 12, G12D was the most common mutation (37.8%), followed by G12C (13.3%), G12S (8.9%) and G12V (4.4%). Within codon 13, G13D was the most common (22.3%). Previous Moroccan studies showed similar results (Marchoudi et al., 2013; Dehbi et al., 2019; Houssaini et al., 2020). However, El Agy et al. revealed a lower rate of G13D mutation in codon 13 (El Agy et al., 2021). Our findings showed that mutation rates in exon 3 and 4 were 15.6% and 13.3%, respectively, which consist with previous Moroccan studies (Dehbi et al., 2019; Houssaini et al., 2020) but higher than others (Dehbi et al., 2019; El Agy et al., 2021). In the NRAS gene, the majority of the mutations are at exon 2 within 57.1%. G13A and Q61H were the most common mutations and were found with 42.9% and 28.5% respectively. In previous Moroccan studies, the most common NRAS mutations were Q61K (2.6%) and Q61R (1.8%) (Dehbi et al., 2019). KRAS Concurrent mutations were identified in 6.7% including, 2.3% patients with two concurrent mutations (Q61R, Q61H), and 4.4% with three concurrent mutations (Q61R, Q61H, Q61L) in codon 61. NRAS mutations have been previously reported to have an association with KRAS wt and are generally found to be mutually exclusive with KRAS mutations. In our study, 4.3% (2/46) KRAS wt was identified with mutations in NRAS gene and 13% mutations concomitantly in KRAS and NRAS genes. Our

findings suggest that multiple mutations can occur in the same codon or different codons.

KRAS, NRAS and BRAF mutations in North Africa
Studies of CRC in North Africa (including Morocco, Algeria, Tunisia, and Lybia) have shown striking differences in full RAS and patterns. In Algeria, KRAS gene mutations were identified in 31.3% and 51.2% of CRC patients (Boudida-Berkane et al., 2016; Mazouzi et al., 2017) with 94.5% of mutated cases in exon 2 (Mazouzi et al., 2017). Codon 12 was the most common KRAS mutation and the most frequently found mutation was G12D ((Boudida-Berkane et al., 2016; Mazouzi et al., 2017). KRAS exon 2 mutations were more frequent in the right colon 40.6% vs. 25% in the left colon (Boudida-Berkane et al., 2016). Outside exon 2, mutations in exon 3 have been identified in 3.5% of tumor cases (Boudida-Berkane et al., 2016). Additionally, 2.3% of CRC tumors harbor NRAS mutations (Mazouzi et al., 2017). BRAF mutations were identified in 4.9% of the tumors patients with CRC. V600E mutation was found in proximal colon in 60% (3/5) tumors in patients with older age >50 years (Boudida-Berkane et al., 2016). In Tunisia, the mutation frequency for the KRAS gene has been reported to be in the range of 23.1 % to 75.2%, dominated by those in exon 2 (Sammoud et al., 2012; Karim et al., 2011; Ines et al., 2014; Jouini et al., 2019; Aissi et al., 2013; Ouerhani et al., 2013). Most exon 2 mutations were detected in codons 12 and G12V and G12D were the most dominated KRAS mutations (Sammoud et al., 2012; Jouini et al., 2019; Aissi et al., 2013; Ouerhani et al., 2013). Mutations outside the KRAS exon2 presented 13.4% of mutated cases and almost a third (28.8%) of KRAS exon 2 wt mCRC (Jouini et al., 2019). Among those, 69.3% carried mutations in NRAS exons2, 3 and 4 and 30.7% in KRAS exons3 and 4 (Jouini et al., 2019). Mutations in BRAF have been found in ~8% (4/48) of mCRC (Bougatef et al., 2008). In Libya, KRAS codon 12/13 mutations were present in 38.2% (13/34) of the CCR patients. The most frequent mutation of codon12 was G12D (46.1%), followed by G12V (17/77, 22.1%) and by G12V (30.8%) and at codon13 were G13D (7.7%) (Aboudabous et al., 2021).

Our findings showed that the KRAS mutation rate was different with more studies that therefore the geographic location and the race/ethnicity backgrounds can influence the KRAS mutation occurrence in North Africa. However, information about ethnicity-based KRAS genotype differences in North Africa is not established. Worldwide, a persistent finding in the literature is the substantial variation in KRAS mutation frequency between race/ ethnic groups. In Africans, the frequency of CRC with KRAS mutations was 21% (Abdulkareem et al., 2012). An analysis of the relationship between mutations and race revealed the predominance of the wt in the Asian group (51.4%). In Europeans, the KRAS gene mutation was more often detected (54.4%) CRC patients (Smagulova et al., 2020). Colon cancers in Asians had a lower rate of KRAS mutations than blacks or whites (Yoon et al., 2015). The finding of novel KRAS and BRAF gene mutations in cancerous tissues obtained from Saudi CRC patients

were typical of those observed elsewhere (Rasool et al., 2021). In Turkey, the mutation frequency for the KRAS mutation gene has been reported to be in the range of 11–49.1% (Akkiprik et al., 2008; Demiralay et al., 2012; Selcukbiricik et al., 2013; Ozen et al., 2013; Baskin et al., 2014). Turkey is known from the geographical location between Europe, the Middle East and the Caucasus region. Thus, Turkey is comprised of many ethnic groups with European, Middle Eastern, Caucasian or Asian origins. The KRAS mutation prevalence may also vary in the same population. Studies from Japan with homogeneous ethnic groups found a wide range of KRAS mutation frequencies (9–71%) (Nishiyama et al., 2002; Mitomi et al., 2003). Studies with heterogeneous ethnic groups also reported various frequencies of KRAS mutation. In the US, CRC tumors from Non-Hispanic Black (48%) or Hispanic patients (44%) carried a greater KRAS mutation rate when compared with Non-Hispanic White (39%) or Asian or Pacific Islander patients (37%) (Leah 2021). The assessment of the impact of KRAS mutation within each race/ethnic group, comparing patients with KRAS mutation vs. wt KRAS on cause-specific survival risk show a 7% risk increase for Non-Hispanic White and a 15% risk increase for Non-Hispanic Black (Bien et al., 2021).

The aspects that seem to play an important role in the incidence of the KRAS gene mutations in CRC in North Africa are lifestyle and dietary patterns. In Morocco, An inverse association between physical activity and CRC risk and a positive association between sedentary behavior and rectal cancer risk has been established recently (Hatime et al., 2022). Furthermore, a marked shift was documented in dietary habits and nutritional intake as part of the nutritional transition. This was associated with an increase in overweight and obesity. According to the body mass index, 29.6% of women were overweight and 15.4% were obese (Barich et al., 2018). These prevalences were considered higher than those reported in the 2003-2004 survey.

The major pattern of dietary habits and nutritional intake includes a large increase in the consumption of high calorie diets and fatty foods in the Moroccan population. Consumption of high amounts of these foods was associated with increased body weight, BMI, and risk of overweight and obesity. High weight-to-height ratio and BMI and obesity were related to increased odds of the KRAS gene mutations (Dolatkhah et al., 2018). Furthermore, significant associations were found between the highest intakes of red meats, cold meats, sausages and the risk of CRC in a case-control study on dietary risk factors for CRC in Moroccan population (Imad et al., 2020. Another study found a significant relationship between the consumption of red meat and colon cancer (OR = 1.23, 95% CI = 1.05–1.44) and CRC risk (OR = 1.14, 95% CI = 1.02–1.27) in patients have been established (Douala et al., 2020). High levels of animal protein, acrylamide foods, and low levels of vitamin A consumption have been shown to be associated with increased risk of CRC tumors with KRAS mutations in patients from Morocco (El Asri et al., 2020). El Asri et al., showed that an increase in the consumption of

Polyunsaturated Fatty Acids (PUFA) above 16.9 g/day was associated with an increase in the presence of KRAS mutations (OR = 2.48, 95% CI = 1.22–4.96) as compared to the reference group whose consumption was less than 16.9 g in CRC Moroccan patients (El Asri et al., 2022). A high intake of PUFA, in particular linoleic acid, may be an important dietary risk factor for KRAS mutated colon tumors, possibly by generating G>A transitions or G>T or G>C transversions in the KRAS oncogene (Brink et al., 2003).

In Algeria, occupational exposures showed a significant link to an increased risk of CRC, as did obesity, alcohol consumption, and passive smoking. Yogurt, cereals, sugar, butter, and margarine consumption were significant protective factors, while cheese, dried fruits, red meat, juice, and fizzy drink consumption was associated with increased CRC risk (Negrichi et al., 2021).

In Tunisia, the CRC incidence has increased markedly from 1994 to 2009, and it is suggested that if no interventions are implemented it is going to double by 2024. It is relevant to highlight that an upward trend in obesity largely explains the increasing incidence of CRC in the Tunisian population. The trend of the incidence of obesity had increased from 10.9% in 1998 to 26.9% in 2016 (El Ati et al., 2007) and 80% of Tunisians aged over 15 years did not consume enough fruits and vegetables per day (Saidi et al., 2019). High total day meat consumption (> 100 g) was significantly associated with a high risk of CRC compared to low consumption (< 50 g) in the Tunisian population (Gharbi et al., 2020).

Limitations

Our study had some limitations. It was a retrospective study and the number of patients was relatively small. It is recommended that future studies be undertaken with larger samples to better understand the frequency and the contribution of the KRAS, NRAS, and BRAF genotype in Morocco CRC patients. In addition, other oncogenic mutations, such as KRAS exon 3 (codons 59, 60, 61), KRAS exon 4 (codons 119, 146, 147) were not assessed. Despite these limitations, our findings may help in implementing effective strategies for RAS (KRAS and NRAS) testing in Moroccan CRC patients.

In conclusion, RAS proteins and their pathway play a causal role in different human cancer, as oncogenic drivers, prognostic and predictive markers, and possible targets. The high prevalence of RAS (KRAS and NRAS) mutations across cancer types makes it an interesting therapeutic target (Cefalì et al, 2021). In CRC, screening for KRAS and NRAS mutations is extremely important predictive and prognostic markers for anti-EGFR monoclonal antibody therapy. In our study RAS (KRAS and NRAS) mutations were identified in 57.5% (56.2% in KRAS and 8.8% in NRAS), indicating that RAS mutation testing is crucial for targeted therapy management in CRC in Morocco. Furthermore, G12C mutation was detected in 13.4% of all KRAS mutated patients. This finding provides an indication of the size of the population that could benefit from treatment with mutant-specific inhibitors. The Code Break 100 trial revealed the potent antitumor effects of AMG510, a novel KRAS G12C inhibitor, against KRAS

G12C-mutant solid tumors, including mCRC (Hong et al., 2020).

Abbreviations
BRAF: B-Raf proto-oncogene
COSMIC: Catalogue of Somatic Mutations in Cancer
CRC: Colorectal Cancer;
DNA: Deoxyribonucleic Acid
EGFR: Epidermal Growth Factor Receptor
FFPE: Formalin-Fixed Paraffin-Embedded
GDP-bound: Guanosine Bi-Phosphate-bound
GTPase: Guanosine Tri-Phosphate ase
GTP-bound: Guanosine Tri-Phosphate-bound
HCAs : Heterocyclic Amines
KRAS : Kirsten Rat Sarcoma
LOD: Limits of detection
MAPK pathway: Mitogen-Activated Protein kinase pathway
NRAS : Neuroblastoma Rat Sarcoma
PAHs: Polycyclic Aromatic Hydrocarbons
PCR: Polymerase Chain Reaction
PUFA: Polyunsaturated Fatty Acids
RAS: Rat Sarcoma; wt: Wild-type.

Author Contribution Statement

SB, FB and AL have conceived the study, exploited data, coordinated and drafted the paper. FH, TB, CH, TM, and RT participated in the designed. SB, AL, MJ, SE, WB, and BEM generated data and involved in data analyses. FE, IAL, MO, MI, KE, ND, and YS have read and agreed to the published version of the manuscript. All authors have read and agreed to the published version of the manuscript

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Ethics approval and consent to participate
Not applicable, the results obtained are part of the normal diagnostic procedure within the hospital for the clinico-pathological evaluation in order to decide on the treatment protocol and the prognosis of the patients.

Availability of data and material
Data involving participants or patients cannot be publicly shared. Individual requests for further information on the study can be sent to the corresponding author.

Conflicts of interest
The authors declare no conflict of interest.

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