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MOLECULAR PROFILING OF COLORECTAL CANCER IN A MOROCCAN COHORT USING TARGETED NEXT GENERATION SEQUENCING

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Abstract

Objective: To determine the mutational spectrum and microsatellite instability (MSI) status in colorectal cancer (CRC) patients in Morocco, and to examine associations between the clinical characteristics.**Methodology**: This prospective study was conducted on 55 patients diagnosed with colorectal adenocarcinoma at the National Institute of Oncology in Rabat. DNA was extracted from FFPE tumor tissues and sequenced using the Oncomine™ Colorectal and Pancreatic Cancer Research Panel. Immunohistochemistry was performed to assess MSI status. Statistical analyses, including correlations between mutations and clinicopathological parameters, were conducted. **Results**: The study found that APC (45.5%), TP53 (43.6%), KRAS (21.8%), ARID1A (20%), PIK3CA (14.5%) and BRAF (12.7%) were the most common mutations. MSI-high status was found in 9.1% of patients, all of whom were ≤60 years old. Statistical analysis revealed a significant correlation between MSI-high status and age ($p = 0.023$), with no correlation found between mutations and other clinicopathological parameters. **Conclusion**: This study provides one of the first comprehensive insight into the mutational landscape of colorectal cancer (CRC) in Morocco, demonstrating a significant associations.The findings highlight the importance of next-generation sequencing (NGS) in guiding personalized treatment strategies for CRC and underscore the need to improve access to molecular diagnostics in Morocco.

Keywords: Colorectal Cancer, Next-Generation Sequencing, Somatic Mutations, Molecular Diagnostics, Microsatellite Instability, Morocco

Introduction

Colorectal cancer (CRC) constitutes an increasing worldwide health threat. According to WHO's GLOBOCAN 2022[1], colorectal cancer (CRC) ranks as the third most prevalent cancer and the second most common cause of cancer mortality globally, with around 1.93 million new cases and 935.000 fatalities recorded in 2022. In Morocco, colorectal cancer is among the top five cancers, with a rising prevalence, particularly in younger people[2].

In this context, understanding the molecular characteristics of CRC within the Moroccan population is of particular importance, given the limited availability of national and regional genomic data.

Colorectal cancer (CRC) exhibits genomic heterogeneity, influenced by mutations in pivotal oncogenes and tumor suppressor genes such as KRAS, NRAS, BRAF, APC, TP53, and PIK3CA, which impact essential pathways affecting cell proliferation, survival, and apoptosis. Mutations in KRAS and NRAS stimulate the RAS/MAPK pathway, favoring proliferation and conferring resistance to anti-EGFR treatment. BRAF V600E affects MAPK signaling, associated with a more severe prognosis [3]. APC mutations stimulate WNT/β-catenin signaling, essential for tumor initiation [4]. TP53 mutations compromise DNA repair mechanisms, resulting in higher genomic instability and increased aggressiveness [5]. PIK3CA mutations stimulate the PI3K/AKT pathway [6], facilitating survival and resistance to treatment. These recurrent Mutations play a pivotal role in understanding the biology of colorectal cancer (CRC) and in the development of personalized therapeutic approaches.The recent advancement in next-generation sequencing (NGS) has transformed the simultaneous detection of multiple mutations, including those in FFPE material, enhancing diagnostic and treatment strategies efficiently. This study aimed to describe the mutational landscape of colorectal cancer in a Moroccan cohort utilizing NGS and to investigate associations that might exist between clinicopathological features.

Methods

Study design and sample collection

This is a prospective study conducted at the National Institute of Oncology in Rabat, Morocco, between March 2023 and May 2025. A total of 60 patients diagnosed with colorectal adenocarcinoma were included. Tumor samples were retrieved from archived formalin-fixed paraffin-embedded (FFPE) tissue blocks stored in the pathology department.

Histopathological evaluation and tumor enrichment

For each case, an H&E-stained slide was reviewed by an experienced pathologist to identify the tumor region and assess tumor cellularity. This histological evaluation guided the precise selection of the tumor-rich area and determined the number of 5–10 µm FFPE sections required for DNA extraction.

DNA Extraction

Genomic DNA was extracted from selected FFPE tissue sections using the KingFisher™ Duo automated purification system [7]. DNA concentration and purity were evaluated using the Qubit™ Fluorometer [7]. Only samples meeting quality control criteria were included in the sequencing step.

Next-Generation Sequencing (NGS)

Targeted sequencing was performed using the Oncomine™ Colorectal and Pancreatic Cancer Research Panel (ORCP) [7], which enables the detection of mutations in 24 genes frequently altered in colorectal and pancreatic cancers. These 24 genes include: APC, ARID1A, CDKN2A, FBXW7, MLH1, MSH2, MSH6, MYC, PIK3CA, PMS2, POLE, PTEN, RNF43, SMAD4, SOX9, TCF7L2, TP53, BRAF, CTNNB1, ERBB2, ERBB3, GNAS, KRAS, NRAS [10]. Library preparation and sequencing were carried out according to the manufacturer's protocol using the Ion S5™ platform [7]. Raw sequencing data were processed and analysed using Torrent Suite™ for alignment and variant calling. For variant annotation and classification, Oncomine Reporter™ software was employed. The results were reviewed and validated by a molecular biologist and a pathologist to ensure accuracy and clinical relevance.

A minimum sequencing depth threshold of ≥500× was required for variant analysis.

Variants were considered only if they had a variant allele frequency (VAF) ≥5%.

Only pathogenic and likely pathogenic variants, as classified according to established databases and the Oncomine Reporter™ annotation system, were included in the final analysis. Variants of uncertain significance (VUS) were excluded.

Quality control parameters included DNA integrity assessment, library quality metrics, and sequencing run performance indicators (including read depth, uniformity, and on-target rate), following manufacturer recommendations.

MSI/MSS status determination

MSI was assessed by immunohistochemistry (IHC) using Dako antibodies and an Autostainer, targeting MSH2, MSH6, PMS2, and MLH1 proteins. An experienced pathologist evaluated staining and controls. Loss of expression in one or more proteins indicated MSI-high (MSI-H), while normal expression indicated microsatellite stable (MSS) or MSI-low. [8]

Statistical Analysis

Clinicopathological features, including tumor differentiation, histology, tumor location, and gender, were reported as frequencies. Age was categorized into two groups using 60 years old as a cutoff. Associations with clinicopathological features were tested using chi-square (χ^2) tests; Fisher's exact test was used when expected counts were few (less than 5). A p-value < 0.05 was considered significant. Analyses were performed with SPSS 25. 0.

Ethical approval statement and patient consent

The protocol was authorized by the Ethics Committee of the Faculty of Medicine in Rabat (CERB 103-25). The research was conducted in accordance with the ethical standards established in the 1964 Declaration of Helsinki and its subsequent amendments or equivalent ethical guidelines. Data were obtained from the Department of Anatomical Pathology at the National Institute of Oncology in Rabat, and all participants provided informed consent for their use.

Results

A total of 60 Next-Generation Sequencing (NGS) assays were performed on colorectal cancer patients, with 5 excluded due to poor DNA quality, leaving 55 patients for analysis. The patients were divided into two age groups: ≤60 years (n=28) and >60 years (n=27). The mean age was 44.2 ± 10.1 years for the ≤60 years group and 69.5 ± 7.8 years for the >60 years group. Males made up 60% of the population, with 33 males and 22 females.
Regarding tumor location, 12.7% of patients in the ≤60 years group and 16.4% in the >60 years group had tumors in the right colon, while 18.2% of the ≤60 years group and 20% of the >60 years group had tumors in the left colon. Rectal tumors were seen in 16.4% of ≤60 years and 10.9% of >60 years.
Adenocarcinoma was the predominant histological type (85.5%), followed by mucinous adenocarcinoma (10.9%). Tumor differentiation revealed that 34.5% of tumors were well differentiated, 56.4% were moderately differentiated, and 9.1% were poorly differentiated.
For MSI status, 90.9% of patients were MSS, with a higher prevalence in the group aged over 60 years. Only 9.1% of patients were MSI, all in the ≤60 years group.
A statistically significant correlation was found between age and MSI status (P=0.023), with all MSI-positive patients being in the ≤60 years group. No significant differences were found for other variables (Table I).

Table I: Demographic and Clinical Distribution

Characteristics	≤60 years (n=28)	>60 years (n=27)	Total (%)	P-value
Age				
- Mean ± SD	44.2 ± 10.1	69.5 ± 7.8	-	-
- Range	21–60	61–83	21–83	
Sex				
- Male	16 (29.1%)	17 (30.9%)	33 (60%)	0,666
- Female	12 (21.8%)	10 (18.2%)	22 (40%)	
Tumor Location				
- Right colon	7 (12.7%)	9 (16.4%)	16 (29.1%)	0,856
- Left colon	10 (18.2%)	11 (20.0%)	21 (38.2%)	
- Rectum	9 (16.4%)	6 (10.9%)	15 (27.3%)	
- Unspecified colon	2 (3.6%)	1 (1.8%)	3 (5.5%)	
Histological Type				
- Adenocarcinoma	24 (43.6%)	23 (41.8%)	47 (85.5%)	0,990
- Mucinous adenocarcinoma	3 (5.5%)	3 (5.5%)	6 (10.9%)	
- Others	1 (1.8%)	1 (1.8%)	2 (3.6%)	
Differentiation				
- Well differentiated	9 (16.4%)	10 (18.2%)	19 (34.5%)	0,922
- Moderately differentiated	16 (29.1%)	15 (27.3%)	31 (56.4%)	
- Poorly differentiated	3 (5.5%)	2 (3.6%)	5 (9.1%)	
MSI Status				
- MSS	23 (41.8%)	27 (49.1%)	50 (90.9%)	**0,023**
- MSI	5 (9.1%)	0 (0%)	5 (9.1%)	

The Figure illustrates the frequencies of mutated genes in a cohort of 55 colorectal cancer patients. *APC* and *TP53* are the most commonly mutated genes, identified in 25 (45.5%) and 24 (43.6%) patients, respectively. Mutations in *KRAS* and *ARID1A* were identified in 12 patients (21.8%) and 11 patients (20.0%), respectively. Some genes exhibit reduced mutation counts and frequencies (Figure 1).

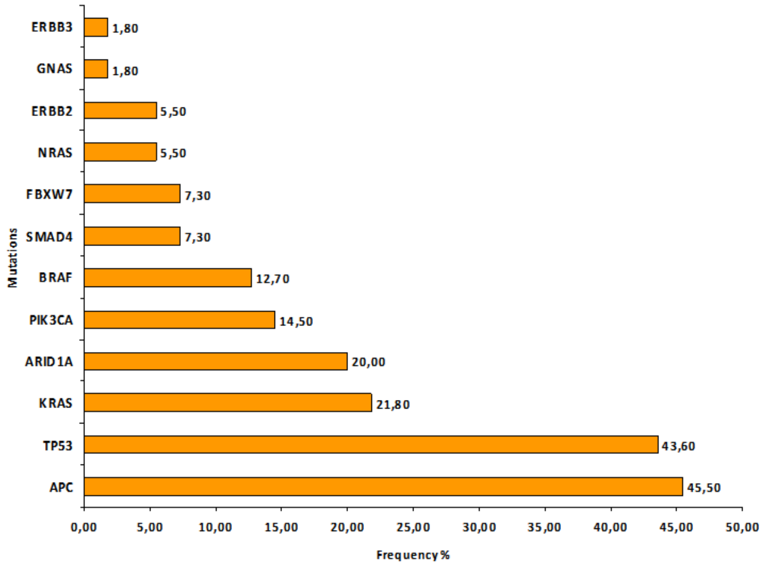

Figure 1: Frequencies of mutated genes among 55 patients

Discussion

The findings of this study highlight the significance of age-related differences in colorectal cancer (CRC) biology. The prevalence of MSI (9.1%) in our cohort aligns with international reports [9], also, younger patients (≤60 years) were found to exhibit a higher prevalence of microsatellite instability (MSI), a feature commonly associated with genetic predispositions such as Lynch syndrome [10]. This is consistent with recent studies that suggest MSI is more prevalent in younger CRC patients [11]. MSI-high status may also have important clinical implications, particularly in relation to prognosis and potential eligibility for immune checkpoint inhibitor therapies. Adenocarcinoma remains the predominant histological type, in line with global CRC trends [12]. The absence of significant differences in tumor differentiation across age groups suggests that factors beyond histological features, such as genetic mutations, may drive CRC progression [13]. These results further emphasize the need for personalized treatment strategies, particularly given the molecular heterogeneity observed across different patient subgroups [14]. Future studies with larger cohorts and comprehensive molecular profiling are essential to refine these insights and improve patient outcomes [15].

This study examined 55 Moroccan colorectal cancer patients to identify prevalent genetic mutations and their clinical associations. Mutations in the APC (45.5%) and TP53 (43.6%) genes indicate their significant role in colorectal carcinogenesis, aligning with findings in international literature [16].

The frequency of KRAS mutation in our cohort is 21.8%, which is lower than the international reports, typically varying between 30% to 48% [17]. The difference can be attributed to the limited size of our sample. Additional factors such as population-specific genetic variability and potential methodological differences may also contribute to this discrepancy. BRAF mutation, found in 12.7% of patients, contrasting with previous studies [18] using the PCR technique, confirmed the frequency and characteristics of BRAF mutations, including the V600E variant, in line with global data.

In our cohort of colorectal cancer patients, the frequencies of mutations in the ARID1A (20%) and PIK3CA (14.5%) genes confirm their crucial role in tumor progression and resistance to targeted inhibitors. The results are consistent with findings that promote the role of genomic alterations, such as ARID1A and PIK3CA, in the molecular classification and progression of colorectal cancer [19]. No pathogenic POLE mutations were identified in our cohort (0%), consistent with the low prevalence reported in the literature (1–3%) [20]. Additionally, the mutations in SMAD4 and FBXW7, noted at 7.3%, align with frequencies documented in the literature, where these alterations are linked to metastatic progression and cell cycle dysregulation [21].

Previous studies have largely focused on a limited set of biomarkers, particularly KRAS, NRAS, BRAF, and microsatellite instability (MSI). While these markers are clinically relevant, such an approach provides only a partial view of the molecular landscape. In contrast, the present study incorporates a broader spectrum of genetic alterations, enabling a more comprehensive characterization of the mutational profile and a more complete understanding of disease biology. Furthermore, these findings underscore the critical importance of comprehensive molecular profiling in guiding precision oncology, particularly in the context of high-risk mutations associated with therapeutic resistance. This highlights the clinical relevance of these biomarkers for optimizing patient stratification and informing targeted treatment strategies.

This study has several limitations that should be considered. First, the relatively small sample size reflects the high cost of next-generation sequencing (NGS) in Morocco, which is not currently supported by the public healthcare system. This financial constraint limits patient access to molecular testing and restricts the ability to assemble larger, statistically robust cohorts.

In this context, the statistical analyses performed in this study should be considered exploratory in nature. The limited sample size, particularly the small number of MSI cases (n=5), also precluded the use of multivariate analysis, as such modeling would lack statistical robustness. Additionally, there is a potential risk of type II error, meaning that some true associations may not have been detected due to insufficient statistical power.

In addition, MSI status was assessed solely by immunohistochemistry without complementary molecular analyses, such as MLH1 promoter methylation testing or germline testing for Lynch syndrome. Consequently, the distinction between sporadic MSI cases and hereditary forms could not be fully established. Furthermore, the lack of longitudinal clinical outcome data and the lack of tumor mutational burden (TMB) analysis limits the ability to evaluate the prognostic and therapeutic implications of the identified mutations. Despite these limitations, this study provides valuable preliminary insights into the mutational landscape of colorectal cancer in a North African population and lays the foundation for future large-scale studies incorporating comprehensive molecular and clinical data

Conclusion

This study underscores the critical role of NGS in advancing personalized medicine for colorectal cancer patients in Morocco. Addressing the financial barriers and expanding access to these technologies are essential to enable larger-scale analyses and ultimately enhance patient care across the country.

Abbreviation List

APC	:Adenomatous polyposis coli
TP53	:Tumor protein p53
KRAS	:Kirsten rat sarcoma viral oncogene homolog
NRAS	:Neuroblastoma RAS viral oncogene homolog
BRAF	:v-Raf murine sarcoma viral oncogene homolog B1
ARID1A	:AT-rich interaction domain 1A
POLE	:DNA polymerase epsilon catalytic subunit
SMAD4	:SMAD family member 4
FBXW7	:F-box and WD repeat domain containing 7
ERBB2	:Erb-B2 receptor tyrosine kinase 2
ERBB3	:Erb-B2 receptor tyrosine kinase 3
GNAS	:GNAS complex locus (Guanine nucleotide-binding protein)
NGS	:Next-generation sequencing
FFPE	:Formalin-fixed, paraffin-embedded
MSI	:Microsatellite instability
MSS	:Microsatellite stable
IHC	:Immunohistochemistry
MAPK	:Mitogen-activated protein kinase
PI3K/AKT	:Phosphoinositide 3-kinase/Protein kinase B pathway
WNT	:Wingless/Integrated signaling pathway
TGF-β	:Transforming growth factor beta
EGFR	:Epidermal growth factor receptor
PIK3CA	:Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha

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