

Introduction

In Morocco, colorectal cancer (CRC) is considered a major public health issue taking into account its burdensome management. It is the third most commonly occurring cancer in men and the second most commonly cancer in women¹. Mutations in full RAS gene (KRAS and NRAS genes) often result in constitutive activation of RAS protein in the epidermal growth factor receptor (EGFR) signaling pathway. Mutations in full RAS gene, particularly in exon 2, 3 and 4 of KRAS gene and in exon 3 and 4 of NRAS gene have been identified as predictors of resistance to anti-EGFR targeted therapy in patients with metastatic CRC (mCRC)². In our study, we have analyzed DNA from 80 FFPE specimens obtained from newly diagnosed CRC patients to assess the frequency and distribution pattern of KRAS and NRAS gene mutations and their association with clinic-pathological features

Material and Methods

DNA was extracted from FFPE tissue using the QIAamp® DNA FFPE Tissue kit (Qiagen). RAS mutations were assessed through pyrosequencing assays using KRAS Pyro® kit 24.V1 and RAS-Extension Pyro® kit 24.V1 (Qiagen) and carried out in the PyroMark-Q24 instrument (Qiagen). The target sequence covering the polymorphic site was amplified with one of the specific biotinylated primers. The pyrosequencing results were analyzed using the PyroMark-Q24 version 2.0.6 software (Qiagen), which identifies the presence of a specific mutation and its percentage. Manufacturer-supplied LOD thresholds were used to call a mutation for LOD studies (\geq % LOD is positive).

Results

KRAS and NRAS mutations were identified in 56.2% and 8.8%, respectively. In KRAS gene, the majority of the mutations are at exon 2 within 93.3%. The mutation rates of exon 3 was 15.6% and of exon 4 was 13.3%. G12D and G13D were the most prevalent mutation (37.8% and 22.3%). In KRAS codons, concurrent mutations were identified in 6.7% cases including 2.3% with two distinct mutations in codon 61, which suggest that multiple mutations can occur in the same codon or different codons. In NRAS gene, the mutation rates of exon 2, 3 and 4 were 57.1%, 28.6%, 14.3%, respectively. G13A and Q61H were the most common mutations and were found with 42.9% and 28.5%, respectively.

Discussion & Conclusion

RAS gene testing have become an important part for clinical management of CRC patients. In our study, KRAS and NRAS gene mutations occur more frequently in CRC patients and these mutations could guide better-tailored therapy for CRC. In Morocco that is witnessing an economic advancement, the adoption of a Western life style and of dietary habits characterized by higher intake of meat, fat and total calories, along with increasing life expectancy and population growth, herald a remarkable increase in the burden of CRC and it can be anticipated that the number of KRAS-mutant CRC is considerable. Therefore, determining the KRAS mutational status of tumor samples has become an essential tool for managing patients with CRC

References

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- [2] B. Zhao, W. Lu, Q. Hong, et al., Mechanisms of resistance to anti-EGFR therapy in colorectal cancer, Oncotarget 8 (2017) 3980-4000.