KRAS/NRAS/BRAF Mutation Rate in Saudi Academic Hospital Patients With Colorectal Cancer

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Abstract

Background: KRAS/NRAS/BRAF mutations are prognostic and predictive molecular biomarkers for colorectal cancers (CRCs). CRC has different frequencies in the population for mutations such as KRAS, NRAS, and BRAF. The aim of this study is to verify the frequency of the somatic KRAS/NRAS/BRAF mutations in Saudi academic hospital patients diagnosed with CRC and compare it with those estimated at the local and national levels.

Methods: Out of 280 colorectal carcinomas diagnosed between 2018 and 2021 (primary and secondary), 97 (34.6%) were evaluated by Next Generation Sequencing (NGS) for colorectal cancer molecular markers. Four of these failed the PCR amplification, while 93 were successfully tested. KRAS, NRAS, and BRAF mutation rates and clinical pathological characteristics were recorded.

Results: In this retrospective study, almost half of the tested samples were reported to have a clinically significant mutation (46/93 positive calls, while others were triple-negative). We found that the most prevalent mutation in KRAS (45.2%) was followed by NRAS (2.2%) and BRAF (2.2%). KRAS p.G12D accounted for the most frequently resulting variant (17/42, 40.5%). Second in ranking is KRAS p.G12V (6/42, 14.3%).

Conclusion: This study is the first to describe the frequency of triple mutations in the city of Jeddah. The findings are consistent with previous research conducted in the Middle East and other local Saudi centers.

Categories: Internal Medicine, Pathology, Oncology
Keywords: colon cancer molecular pathology, arab population, next-generation sequencing (NGS), colorectal cancer (CRC), kras/nras/braf mutations

Introduction

Colorectal cancer (CRC) incidence has increased due to external risk factors such as diet, obesity, and sedentary lifestyle [1]. It is the third most popular cancer diagnosed in men and the second most popular cancer in women [2]. In parallel to worldwide figures, Arab countries like Saudi Arabia have shown an increase in CRC incidence as well [3]. In 2016, CRC accounted for the most common cancer in Saudi males and the third in Saudi females, following breast and thyroid. The median age at diagnosis was 59 years for male patients and 57 years for female patients. Histologically, most lesions were typical adenocarcinomas, followed by mucinous adenocarcinomas.

Mortality-wise, one of the major reasons for death worldwide is CRC, representing 9.4% of all cancer deaths [1]. While the number of cases in non-metastatic situations has been detected early by screening, addressability, and increased awareness, about 27% of CRC patients in Saudi Arabia were diagnosed at stage IV [4]. As such, in the past 20 years, the focus of the bulk of cancer research has been directed towards advancing therapeutic modalities in the field of metastatic CRC [5,6].

CRC development is mainly driven by genetic mutation and defective cell regulation [7]. Multiple signaling pathways such as RAS-RAF-MAPK, which play an important role in angiogenesis, cell proliferation, and motility, are activated by the accumulation of these mutations, including KRAS, NRAS, and BRAF [8-10].

In the modern age of personalized cancer treatment, the assessment of genetic mutations is a key element. The understanding and predictability of these mutations have revolutionized the treatment of many malignancies recently, improving outcomes as well as patient care [11,12].

The identification of molecular tumor markers predicting the response to treatment is a fundamental element of improved patient management. However, the cost of targeted therapy has been linked to a substantial financial burden for healthcare. Thus, it is extremely important to select appropriate candidates for specific treatment [13].

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Biomarker applications such as KRAS and BRAF for individualized medicine have voiced translation research. A major determinant of panitumumab and cetuximab treatment in colorectal cancer is the presence of mutations in the KRAS gene [14-16]. At present, one of the most common methods for predicting the response to the EGFR inhibitors is to test for some ‘activating’ mutations of the KRAS gene. EGFR inhibitors show minimal effects in tumors harboring KRAS mutations.

As far as is known, BRAF mutations appear to be associated with a dismal prognosis. The most common BRAF alteration (V600E) is responsible for MAPK pathway activation. Multiple tumors harbor BRAF oncogenic mutations. The CRC subgroup, which arose from serrated polyps, has a molecular phenotype described by hypermethylation of CpG in known enhancer gene regions and is named as the CpG island methylated phenotype (CIMP). The serrated pathway is led by BRAFV600E-driven hypermethylation [14]. Thus, the risk of subgroup-specific CRC and disease progression is assessed by advancements in epigenetics.

The rate of KRAS/NRAS/BRAF mutations needs to be further defined in Arab patients [15]. The aim of the study is to investigate the frequency of the triple of somatic KRAS/NRAS/BRAF mutations in an academic tertiary hospital in the city of Jeddah, as it has not been described previously. The data can be used as input features for the development and validation of a model for the prediction and visualization of mutations in machine learning algorithms, such as clinical variants and histological parameters.

Materials And Methods
This retrospective-designed study was conducted at King Abdulaziz University Hospital, Jeddah. Out of 280 patients with sporadic CRC diagnosis (primary or secondary) between October 2018 (when the test was first implemented) and September 2021, 97 tumor tissue samples were subjected to TruSight® Tumor 15 Illumina panel with a combined hotspot mutation test using a 15-gene multiplex platform by Next Generation Sequencing (NGS). After obtaining the unit of biomedical research’s ethics committee approval, sequencing results were retrieved from the hospital’s electronic information system along with their epidemiological data (age, gender, tumor location, stage, and morphology).

For all of these patients, DNA isolation was carried out using formalin-fixed paraffin-embedded tissue (FFPE). All tissue blocks of the FFPE were obtained from the Histopathology Department and the tumor content was thoroughly checked. Five to eight 10-μm sections were used for DNA for the extraction. The Qiagen AllPrep DNA FFPE Kit is isolated with DNA as per instructions from the manufacturer.

Results
The mean age of the reported pathogenic variant cases was 57.3, with no gender bias (the male-to-female ratio was almost 1:1). Out of 97 samples, 93 passed the quality parameters. The detected mutations were identified in 46/93 cases (49.5%), while others had triple-negative results. All the studied alterations (KRAS/NRAS/BRAF) were mutually exclusive. The flagged KRAS calls were 42/93, representing 45.2%, while NRAS and BRAF had two detected variants for each, representing 2.2%. The frequency of KRAS alterations outweighed NRAS and BRAF, as illustrated in Table 1. The most common detected KRAS oncogenic mutation is G12D, followed by G12V (Table 1). Clinical correlation wise, of all mutated tumors, 30 exhibited aggressive behavior (65.2%) such as recurrence, distant metastasis, and poor response to chemotherapy (27 of which were of KRAS pathogenic alterations, 1 NRAS and all BRAF mutated cases). In addition, 37 positively called samples (75.9%) were left-sided, mostly rectosigmoid (54, 2, 1 mutations found in KARS, NRAS, and BRAF, respectively). Overall, 14 cases (15%) were of the mucinous subtype, most of which (9 cases, 64.3%) carried KRAS mutations (Figure 1).
### TABLE 1: Mutation frequencies in colorectal cancer.

| Mutation | Frequency | Percent |
|----------|-----------|---------|
| KRAS G12D | 17 | 18.3 |
| KRAS G12V | 6 | 6.5 |
| KRAS G12S | 4 | 4.3 |
| KRAS G13D | 3 | 3.2 |
| KRAS G12A | 3 | 3.2 |
| KRAS Q61H | 2 | 2.2 |
| KRAS K117N | 2 | 2.2 |
| KRAS A146T | 2 | 2.2 |
| KRAS G12R | 1 | 1.1 |
| KRAS G12C | 1 | 1.1 |
| KRAS G138E | 1 | 1.1 |
| NRAS Q61K | 1 | 1.1 |
| NRAS Q61L | 1 | 1.1 |
| BRAF V600E | 2 | 2.2 |
| Triple negative | 47 | 50.5 |
| Total | 93 | 100.0 |

**Discussion**

CRC is considered one of the most complex cancers as it is classified according to its pathological features. Several studies have shown interesting results in correlating these features or demographic data with gene mutation. Our figures are similar to those that have been documented globally. The current literature shows that activating KRAS mutations predominate in CRC cases (35-45%) with the most frequently detected hotspot codons being 12 and 13 (glycine substituting aspartate p.G12D, p.G13D). In contrast, the mutation rates of NRAS are the lowest (1-3%) [16].
At the pathogenesis level, CRC results from traditional pathways (KRAS/BRAF wild type), alternative pathways (KRAS mutation), and the serrated pathways (BRAF mutation - dMMR), representing 35.4%, 11.6%, and 0.8% of CRC cases in the Middle East, respectively [17]. Similarly, our CRC cases' molecular analysis revealed that the serrated pathway harboring BRAF mutation accounted for a very small percentage (2.2%), which falls behind the adenoma–carcinoma KRAS-mutated pathway (45.2%)

The prevalence of CRC drivers' mutation rates has been slightly variable among different Middle Eastern countries. MD Anderson Cancer Center in Texas published their Arab patients' data describing that KRAS/NRAS/BRAF prevalence were 44.4%, 4%, and 4%, respectively, compared to 48.4%, 4%, and 4%, respectively, in the matched Western population [18]. Another reference laboratory in the United States has published the KRAS mutation frequency in the Middle Eastern countries as follows: Algeria (38.4%), Egypt (27.4%), and Saudi Arabia (34.7%) [19]. In addition, a Lebanese tertiary medical center showed that RAS and BRAF mutation rates were 38.5% and 12.9%, respectively [20]. Furthermore, Elbiejriami et al. from Jordan reported the rate of KRAS mutations in the Jordanian population with CRC is 44% [21].

Our retrospective study revealed that the KRAS mutations are a little higher (45.2%), with left-sided location and disease progression similar to what has been published by other local academic centers. For instance, King Fahad Medical City - Riyadh observed KRAS mutations in 42.2% of patients with CRC, mostly p.G12D, p.G12V, and p.G13D. Observed tumors accounted for 51% of the left hemicolon and 23% of the rectum. 74% of the mutations were reported in patients with advanced CRC. 31% of KRAS-mutated patients had stage IV disease with wide distant metastasis, particularly liver and lung, compared to 19% in the wild-type group [22]. King Saud bin Abdulaziz University for Health Sciences - Riyadh reported their mutation frequency as follows: KRAS (49.6%), NRAS (2%), and BRAF (0.4%). 44.4% of their patients exhibited wild-type tumors [23]. At Tibah University - Madinah, the proportion of patients with RAS mutations was 43%. 91% of these mutations were in KRAS. Codons 12 and 13 were the most detected locations, representing 75% and 20%, respectively, in particular, p.G12D and p.G13D [24]. On follow-up, KRAS and BRAF mutations predicted poor prognosis and survival [25].

Conclusions

On the basis of the existence of biomarkers, emerging molecular markers can be a valuable tool for early identification and prevention of CRC, as well as guiding the therapeutic process with individualized therapy. With the rapid advancement of molecular testing and our growing understanding of CRC and its molecular progression, we may soon enter a new era of personalized therapy in which routinely used biomarkers enable more precise patient treatment. In this analysis, KRAS p.G12D was the most frequently observed variant associated with CRC in the Saudi population. The second in frequency was KRAS p. G12V. Our data are in line with others published locally and regionally. As a result, the current therapeutic option guidelines are applicable to our patients as well. Further research on various populations needs to be conducted in order to obtain a better understanding of their precise position and occurrence.

Additional Information

Disclosures

Human subjects: Consent was obtained or waived by all participants in this study. Unit of Biomedical Ethics - Research Committee issued approval 405-21. After obtaining the unit of biomedical research – ethics committee approval, sequencing results were retrieved from the hospital electronic information system along with their epidemiological data (age, gender, tumor location, stage and morphology). Animal subjects: All authors have confirmed that this study did not involve animal subjects or tissue. Conflicts of interest: In compliance with the ICMJE uniform disclosure form, all authors declare the following: Payment/services info: All authors have declared that no financial support was received from any organization for the submitted work. Financial relationships: All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. Other relationships: All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

References

1. Rawla P, Sunakara T, Barsonuk A: Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. Pro Gastroenterol. 2019, 14:89-105. 10.5114/pg.2018.81072.
2. Sung HI, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F: Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021, 71:209-49. 10.3322/canjc.21660.
3. Ibrahim EM, Zeeneldin AA, El-Khodary TR, Al-Gahmi AM, Bin Saadj BM: Past, present and future of colorectal cancer in the Kingdom of Saudi Arabia. Saudi J Gastroenterol. 2008, 14:178-82. 10.4103/1319-3767.42275.
4. Cancer Incidence Report Saudi Arabia 2016. Saudi Health Council. National health Information Center. Saudi Cancer Registry. (2016). Accessed: March 26, 2022; https://nhic.gov.sa/eServices/Documents/2016.pdf.
5. Moghimi-Dehkordi B, Safaee A: An overview of colorectal cancer survival rates and prognosis in Asia. World J Gastrointest Oncol. 2012, 4:71-5. 10.4251/wjgo.v4.i4.71.
6. Wolpin BM, Mayer RJ: Systemic treatment of colorectal cancer. Gastroenterology. 2008, 134:1296-310. 10.1053/j.gastro.2008.02.098
7. Armaghany T, Wilson JD, Chu Q, Mills G: Genetic alterations in colorectal cancer. Gastrointest Cancer Res. 2012, 5:19-27.
8. McCubrey JA, Steelman LS, Chappell WH, et al.: Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. Biochim Biophys Acta. 2007, 1773:1263-84. 10.1016/j.bbamcr.2006.10.001
9. Peyssonnaux C, Eychène A: The Raf/MEK/ERK pathway: new concepts of activation. Biology of the Cell. 2001, 93:53-62. 10.1016/s0248-4900(01)01125-x
10. Calistri D, Rengucci C, Seymour I, et al.: Mutation analysis of p53, K-ras, and BRAF genes in colorectal cancer progression. J Cell Physiol. 2005, 204:484-8. 10.1002/jcp.20310
11. Zhang J, Zheng J, Yang Y, et al.: Molecular spectrum of KRAS, NRAS, BRAF and PIK3CA mutations in Chinese colorectal cancer patients: analysis of 1,110 cases. Sci Rep. 2015, 5:18678. 10.1038/srep18678
12. Bokemeyer C, Van Cutsem E, Rougier P, et al.: Addition of cetuximab to chemotherapy as first-line treatment for KRAS wild-type metastatic colorectal cancer: pooled analysis of the CRYSTAL and OPUS randomised clinical trials. Eur J Cancer. 2012, 48:1466-75. 10.1016/j.ejca.2012.02.057
13. Wei Q, Shui Y, Zheng S, et al.: EGFR, HER2 and HER3 expression in primary colorectal carcinomas and corresponding metastases: implications for targeted radionuclide therapy. Oncology reports. 20111, 25:3-11. 10.3892/or_00001035
14. Vaughn CP, Zobell SD, Furtado LV, Baker CL, Samowitz WS: Frequency of KRAS, BRAF, and NRAS mutations in colorectal cancer. Genes Chromosomes Cancer. 2011, 50:507-12. 10.1002/gcc.20854
15. Siraj AK, Bu R, Prabhakaran S, et al.: A very low incidence of BRAF mutations in Middle Eastern colorectal carcinoma. Mol Cancer. 2014, 15:168. 10.1186/1476-4598-15-168
16. Nguyen HT, Duong HQ: The molecular characteristics of colorectal cancer: Implications for diagnosis and therapy. Oncol Lett. 2018, 16:9-18. 10.3892/ol.2018.8679
17. Beg S, Siraj AK, Prabhakaran S, et al.: Molecular markers and pathway analysis of colorectal carcinoma in the Middle East. Cancer. 2015, 121:3799-808. 10.1002/cncr.29580
18. Al-Shamsi HO, Jones J, Fahmawi Y, et al.: Molecular spectrum of KRAS, NRAS, BRAF, PIK3CA, TP53, and APC somatic gene mutations in Arab patients with colorectal cancer: determination of frequency and distribution pattern. J Gastrointest Oncol. 2016, 7:882-902. 10.21037/jgo.2016.11.02
19. Garawin T, Lowe K, Kafatos G, Murray S: The prevalence RAS and BRAF mutations among patients in the Middle East and Northern Africa with metastatic colorectal cancer. J Clin Oncol. 2016, 34:e15077. 10.1200/JCO.2016.34.15_suppl.e15077
20. Ibrahim T, Saer-Ghora C, Trak-Smayra V, Nadiri S, Yazbeck C, Baz M, Kattan JG: Molecular characteristics of colorectal cancer in a Middle Eastern population in a single institution. Ann Saudi Med. 2018, 38:251-9. 10.5144/0256-4947.2018.251
21. Elbjeirami WM, Sughayer MA: KRAS mutations and subtyping in colorectal cancer in Jordanian patients. Oncol Lett. 2012, 4:705-10. 10.3892/ol.2012.785
22. Bader T, Ismail A: Higher prevalence of KRAS mutations in colorectal cancer in Saudi Arabia: Propensity for lung metastasis. Alexandria J Med. 2014, 50:205-9. 10.1016/j.ajme.2014.01.003
23. Alharbi A, Bin Dokhi H, Almuhaini G, Alomran F, Masuadi E, Alomran N: Prevalence of colorectal cancer biomarkers and their impact on clinical outcomes in Riyadh, Saudi Arabia. PLoS One. 2021, 16:e0249590. 10.1371/journal.pone.0249590
24. Mulla N, Alshebref A, Syed AR, Al-Jahel M: Clinico-pathological study of K-ras mutations in colorectal tumors: a single-center retrospective study of 51 patients in Madinah, Saudi Arabia. Cureus. 2020, 12:e9978. 10.7759/cureus.9978
25. Alkharji H: Clinicopathological features and predictive factors for colorectal cancer outcome in the Kingdom of Saudi Arabia. Oncology. 2017, 92:75-86. 10.1159/000450857