Since 1987
REVIEW OF CLINICAL PHARMACOLOGY AND PHARMACOKINETICS, INTERNATIONAL EDITION | 38 (Sup2):73-76 (2024) Rcpp

Received: 29 February 2024 | **Accepted**: 17 April 2024 | **Published**: 5 May 2024

Open Access | Research

Molecular study of *KRAS* mutations in Iraqi patients with gastrointestinal tract cancer

Hajir B. Aljaryan[1], Ataa Khalil Al-Shamari[1,*], Taif Hussein Al-Ameedy[1], Amal Talib Al Sa'ady[1], Samir Azzat Malik Hussain[2]

[1]Department of Clinical Laboratory Sciences, College of Pharmacy, University of Babylon, Hillah, Iraq
[2]College of Pharmacy, Al-Mustaqbal University, Hillah, Iraq

***Corresponding author:** Ataa Khalil Al-Shamari, Department of Clinical Laboratory Sciences, College of Pharmacy, University of Babylon, Hillah, Iraq; Tel.: +964-(0)7722236034
E-mail: pharm.ataa.khalil@uobabylon.edu.iq

Abstract

Gastrointestinal cancers, including stomach, liver, oesophageal, pancreatic, and colorectal cancers, represent more than a quarter of all cancers. Many abnormal gene expressions and dysregulated signalling pathways have been found in human cancer. Cancer often has activating mutations of the KRAS (Kirsten rat sarcoma virus) oncogene. Fifty blood samples from gastrointestinal cancer patients were gathered from the Merjan Teaching Hospital in Babylon, Iraq, and were used for a case-control study in the Oncology Center. According to the results, the most common cancers were found in the colon (29%), followed by the liver (27%), pancreas (19%), stomach (13%), and other (12%). In this work, we evaluated the distribution of *KRAS* mutations across the gastrointestinal tract. Sequencing data revealed a significant regional difference in the frequency of *KRAS* mutations, while the alignment results revealed the presence of six variations in the analysed samples when compared with the referring reference DNA sequences. Six highly interesting nucleic acid polymorphisms were detected in the investigated samples. When combined with additional carcinogenic markers such as the patient sex, age, consistent molecular subtypes, and tumour stage, *KRAS* mutation is not the deterministic carcinogenic factor for gastrointestinal malignancies.

KEYWORDS

polymorphism, KRAS gene, gastrointestinal tract cancer, protein folding, mutation

How to cite: Aljaryan H. B., Al-Shamari A. K., Al-Ameedy T. H., Al Sa'ady A. T., Hussain S. A. M. Molecular study of *KRAS* mutations in Iraqi patients with gastrointestinal tract cancer. *Rev. Clin. Pharmacol. Pharmacokinet. Int. Ed.* 38 (Sup2): 73-76 (2024). https://doi.org/10.61873/UUXZ2461

Publisher note: PHARMAKON-Press stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2024 by the authors.
Licensee PHARMAKON-Press, Athens, Greece.

This is an open access article published under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. INTRODUCTION

Cancer is a leading cause of death worldwide, with gastrointestinal cancers such as colon, pancreatic, and gastric cancer posing significant health threats. Gastrointestinal cancers are a diverse group of malignancies that arise from different sites in the digestive system, each with its unique risk factors, symptoms, and molecular pathology [1]. Many risk factors or causes of cancer development contribute to the changes in a cell that can result in cancer. These risk factors may be intrinsic to an individual, such as sex, age, or genes. However, most risk factors are

external, in the individual's general environment. The interplay between the intrinsic and external factors is the major determinant of an individual's cancer risk [2].

A biomarker is an objectively measured characteristic that describes a normal or abnormal biological state in an organism by analysing biomolecules such as DNA, RNA, proteins, and peptides, as well as biomolecule chemical modifications. Biomarkers are useful in several ways, including measuring the progress of disease, evaluating the most effective therapeutic regimes for a particular cancer type, and establishing long-term susceptibility to cancer or its recurrence [3]. The three human RAS genes (*KRAS*, *NRAS*, and *HRAS*) are the most frequently mutated oncogenes in human cancer, appearing in 90% of pancreatic, 35% of lung, and in 45% of colon cancers. These high occurrences make RAS one of the most important targets in oncology for drug development, In particular, KRas is the isoform prevalently mutated in pancreas, lung, and colon cancer [4]. This case-control study aimed to analyse whether a relationship between the KRAS gene and gastrointestinal tract cancer patients exists.

2. PATIENTS AND METHODS

The current case-control study included 50 samples of patients with gastrointestinal tract cancer and 50 apparently healthy individuals as a control group. Samples were collected from the Merjan Teaching Hospital in Babylon (Iraq) by the Oncology Center. Each patient with cancer was confirmed and diagnosed by an oncologist; for all patients, a complete history was taken, which included age, sex, smoking habits, family history, duration of disease, record of chemotherapy, and type of cancer. This study was undertaken from February 2018 to May 2019. About 50 blood specimens were obtained from patients aged 30 years to 80 years, while the control cases were aged 33 years to 81 years. Genomic DNA was extracted and purified by using a G-spin™ Total DNA Extraction Mini Kit (Intron, Korea) according to the manufacturer's instructions. Conventional polymerase chain reaction tests were undertaken in order to detect the KRAS genome, while gene polymorphisms were genotyped by utilizing the single-strand conformation polymorphism method.

The study's protocol was reviewed and approved by the University of Babylon College of Science ethical committee.

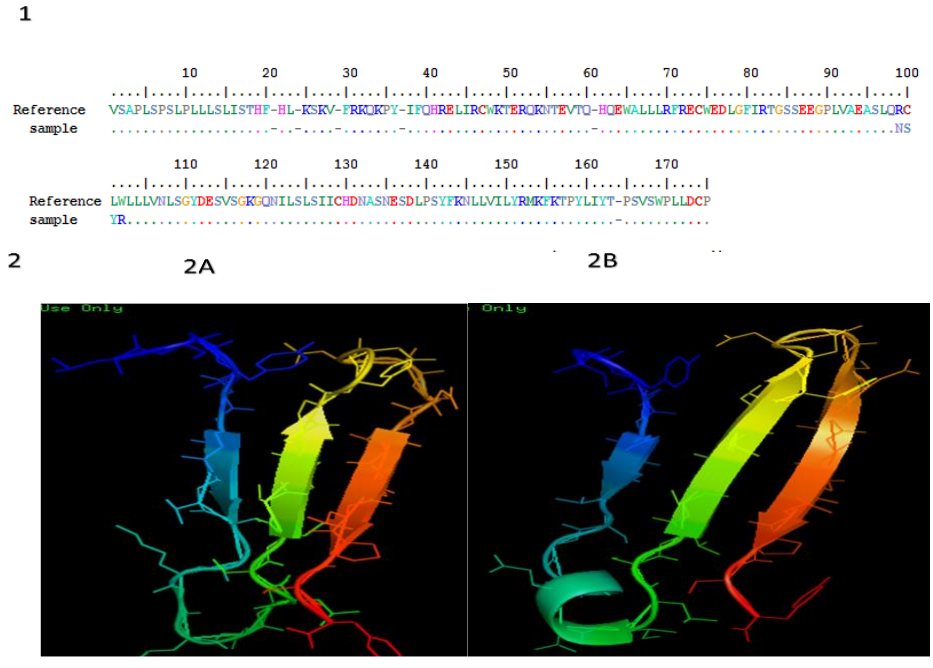

Figure 1. Top panel (1): sequence alignment of amino acids in the K-Ras protein drawn based on the sequence alignments obtained by the BioEdit program version 7.2.5. Bottom panel (2): 3D secondary structures of the K-Ras protein in patients with gastrointestinal cancer: normal (2A) and abnormal (2B)

3. RESULTS

A molecular study of the gastrointestinal tract cancer samples demonstrated that regarding the type of cancer, colon cancer represented 29% of the samples, with liver (27%), pancreas (19%), stomach (13%), and other types (12%) of cancer being represented at lower percentages. The results obtained from the sequenced 532 bp fragments as well as the detailed positions of the observed variations are described in the NCBI reference sequences. The KRAS gene polymorphism revealed six types of substitutions: guanine (G) to adenine (A; G>A) in position 301 of the sequence, G to thymine (T; G>T) in position 302 of the sequence, T to cytosine C; T>C) in positions 303 and 309 of the sequence, G to C (G>C) in all samples in position 304 of the sequence, C to T (C>T) in positions 305 and 306 of the sequence, and T to A (T>A) in positions 307 and 308 of the sequence. When translating the DNA sequence by using by the BioEdit program (version 7.2.5) according to the reference sequence alignment of the K-Ras protein, arginine changed to asparagine, cysteine to serine, leucine to tyrosine, and tryptophan to arginine. This result led to a change in the 3D secondary structure of the protein in patients with gastrointestinal cancer as shown in Figure 1.

4. DISCUSSION

The use of gene alterations in blood in order to track circulating tumour DNA has been attempted for clinical applications. For example, *KRAS* monitoring in colorectal cancer provides a valuable biomarker for diagnosis and the prediction of treatment outcomes. While half of colon cancer samples have a *KRAS* mutation, 90% of pancreatic cancer samples also showed a *KRAS* mutation, thereby suggesting that most pancreatic cancers can be a good candidate for *KRAS* monitoring [5].

KRAS mutations refer to a frequent G>A alteration. The K-Ras protein is a GTPase, which means it converts a molecule called GTP into another molecule called GDP. In this way the K-Ras protein acts like a switch that is turned on and off by the GTP and GDP molecules. To transmit signals, it must be turned on by attaching to a molecule of GTP. The K-Ras protein is turned off when it converts the GTP to GDP. When the protein is bound to GDP, it does not relay signals to the cell's nucleus [6].

The KRAS gene belongs to a class of genes known as oncogenes. When mutated, oncogenes have the potential to cause normal cells to become cancerous [7]. The activation KRAS gene point mutations have been detected in many types of human tumours, as the oncogenic forms of the KRAS gene are prevalent in pancreatic (>80%), colon (40%–50%), and lung (30%–50%) cancers, but are also present in biliary tract malignancies, endometrial cancer, cervical cancer, bladder cancer, liver cancer, and myeloid leukaemia [8]; in fact, most studies support its early involvement in carcinogenesis. Current evidence correlates *KRAS* mutations with increased cell proliferation and apoptosis. Tumours positive for KRAS mutation can harbor hypermethylation-related changes in genome expression, and this can be the cause of concurrent loss of DNA repair proteins. Despite the existence of evidence that the *KRAS* mutation status affects cancer progression, the effects of these mutations on tumour sensitivity to cytotoxic chemotherapies and radiation have only been explored by a few studies [9]. The presence of oncogenic *KRAS* has been found to significantly increase the sensitivity of cells to a novel class of anticancer agents.

Finally, a protein is considered to be misfolded if it cannot achieve its normal native state. This can be due to mutations affecting its amino acid sequence. The misfolding of proteins can trigger the further misfolding and accumulation of other proteins into aggregates or oligomers [10].

ACKNOWLEDGEMENTS

The authors would like to express their thanks to the College of Pharmacy of the University of Babylon, for its support and the resources provided.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

REFERENCES

1. Siegel R.L., Miller K.D., Wagle N.S., Jemal A.: Cancer statistics, 2023. *CA Cancer J. Clin*. 73(1): 17-48 (2023).
DOI: **10.3322/caac.21763**
PMID: **36633525**

2. Xie Y., Shi L., He X., Luo Y.: Gastrointestinal cancers in China, the USA, and Europe. *Gastroenterol. Rep*. (Oxf.) 9(2): 91-104 (2021).
DOI: **10.1093/gastro/goab010**
PMID: **34026216**

3. Huss R.: Biomarkers. In: Atala A., Allickson J.G. (editors): Translational Regenerative Medicine. Amsterdam: Academic Press, 235-241 (2015).
DOI: **10.1016/B978-0-12-410396-2.00019-0**

4. Cox A.D., Fesik S.W., Kimmelman A.C., Luo J., Der C.J.: Drugging the undruggable RAS: mission possible? *Nat. Rev. Drug Discov*. 13(11): 828-851 (2014).
DOI: **10.1038/nrd4389**
PMID: **25323927**

5. Watanabe F., Suzuki K., Ichida K., Takayama Y., Fukui T., Kakizawa N., *et al.*: The clinical significance of *KRAS* monitoring in tumor tissues and blood of patients with pancreatic tumor. *J. Clin. Oncol*. 35(suppl. 4): 334 (2017).
DOI: **10.1200/JCO.2017.35.4_suppl.334**

6. Addissie Y.A., Kotecha U., Hart R.A., Martinez A.F., Kruszka P., Muenke M.: Craniosynostosis and Noonan syndrome with *KRAS* mutations: expanding the phenotype with a case report and review of the literature. *Am. J. Med. Genet*. A 167A(11): 2657-2663 (2015).
DOI: **10.1002/ajmg.a.37259**
PMID: **26249544**

7. (---): KRAS gene. MedlinePlus website (2024).
website: **medlineplus.gov/genetics/gene/kras**

8. Downward J.: Role of receptor tyrosine kinases in G-protein-coupled receptor regulation of Ras: transactivation or parallel pathways? *Biochem. J.* 376(Pt 3): e9-e10 (2003).
DOI: **10.1042/BJ20031745**
PMID: **14656216**

9. Sideris M., Emin E.I., Abdullah Z., Hanrahan J., Stefatou K.M., Sevas V., *et al.*: The role of *KRAS* in endometrial cancer: a mini-review. *Anticancer Res*. 39(2): 533-539 (2019).
DOI: **10.21873/anticanres.13145**
PMID: **30711927**

10. Khan M.V., Zakariya S.M., Khan R.H.: Protein folding, misfolding and aggregation: a tale of constructive to destructive assembly. *Int. J. Biol. Macromol*. 112: 217-229 (2018).
DOI: **10.1016/j.ijbiomac.2018.01.099**
PMID: **29374532**