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RESEARCH ARTICLE

The Relationship Between Kirsten Rat Sarcoma Mutations and Mismatch Repair Status in Colorectal Cancer Patients: A Preliminary Study

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Abstract

Background: Chromosomal instability (CIN) is a key pathway in colorectal cancer (CRC) tumorigenesis, occurring in 80%-85% of cases and leading to aneuploidy. CIN tumors often harbor mutations in APC, KRAS, and TP53, which drive tumor progression. Another crucial pathway, microsatellite instability (MSI), results from DNA mismatch repair (MMR) defects, leading to a hypermutable phenotype. MSI-CRC has distinct clinicopathologic features and a better prognosis than proficient MMR (pMMR) CRC. KRAS mutations, found in approximately 40% of CRC cases, drive tumor progression via the RAS/RAF/MAPK pathway. This study investigates the relationship between KRAS mutations and MMR status in CRC patients. **Methods:** A preliminary cross-sectional study was conducted on CRC patients. KRAS mutations were analyzed using PCR, while MMR status was determined via immunohistochemistry and microsatellite testing. Clinical variables, including cancer stage, tumor category, and location, were analyzed. **Results:** Among 55 patients, 91.3% had pMMR and 8.7% had deficient MMR (dMMR). KRAS mutations were more frequent in pMMR patients (80% of cases). Patients with pMMR and KRAS mutations exhibited aggressive tumors but responded to conventional therapy, whereas dMMR patients with KRAS mutations showed complex molecular profiles, potentially benefiting from immunotherapy. **Conclusion:** KRAS mutations are primarily associated with pMMR in CRC, indicating distinct molecular pathways. Personalized treatment strategies should consider MMR status and KRAS mutations to optimize therapeutic outcomes. Further research is needed to explore their clinical implications.

Keywords: KRAS mutations- mismatch repair- colorectal cancer

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Introduction

Colorectal cancer (CRC) is a major global health concern, with approximately 1.9 million new cases and nearly 1 million deaths annually [1]. In 2020, CRC accounted for 10% of all new cancer diagnoses worldwide. [2]. In Indonesia, CRC ranks among the most prevalent cancers, with 34,189 cases reported, [3, 4] highlighting the need for improved screening, treatment, and management strategies. Given its high incidence, understanding the molecular mechanisms underlying CRC is essential for optimizing patient outcomes [5].

CRC development is a complex, multifactorial process influenced by genetic and environmental factors [6-8]. It often progresses through the adenoma-carcinoma sequence, with key genetic alterations in APC, KRAS, TP53, and DCC playing crucial roles [1]. The two primary molecular pathways in CRC tumorigenesis are chromosomal instability (CIN) and microsatellite instability (MSI) [9]. CIN, present in 80%-85% of CRC cases, (1) is characterized by aneuploidy and mutations

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in genes such as APC, KRAS, and TP53 [10, 11]. MSI, resulting from defects in the DNA mismatch repair (MMR) system, leads to a hypermutable phenotype and distinct clinicopathologic features, often associated with a better prognosis [12-14].

KRAS mutations occur in approximately 40% of CRC cases and are predominantly linked to the CIN pathway [15].

These mutations activate the RAS/RAF/MAPK signaling cascade, promoting tumor proliferation and progression [16]. While KRAS mutations in proficient MMR (pMMR) CRCs are associated with poor response to anti-EGFR therapy, deficient MMR (dMMR) tumors exhibit different molecular profiles, including a higher prevalence of BRAF mutations and potential responsiveness to immunotherapy [17, 18]. Understanding the interaction between KRAS mutations and MMR status is crucial for refining CRC treatment strategies.

Despite extensive global research, limited studies have explored the relationship between KRAS mutations and MMR status in Indonesian CRC patients. Most existing studies have focused on other cancer types or international populations. This study aims to bridge this gap by analyzing KRAS mutations and MMR status in CRC patients in Makassar, providing valuable insights into the genetic landscape of CRC in Indonesia and contributing to more personalized treatment approaches.

Methods

This preliminary study utilized an observational analytic cross-sectional design to investigate the relationship between KRAS mutations and MMR status in CRC patients. The research was conducted at Wahidin Sudirohusodo Hospital and its affiliated teaching hospitals between January and December 2023. The estimated number of participants was calculated using Lemeshow's formula [19, 20]. Patients diagnosed with CRC were selected through consecutive sampling, with inclusion criteria comprising a confirmed pathological diagnosis, age of 18 years or older, and the availability of sufficient tissue samples for genetic analysis. Patients were excluded if they had systemic malignancies, metastases from other cancers, or conditions that could interfere with study participation or prognosis.

Detection of KRAS Gene Mutations

The detection of KRAS gene mutations in this study relied on the technique of Real-Time Polymerase Chain Reaction (RT-PCR), specifically targeting mutations in codons 12 and 13. Sample preparation commenced with the isolation of genomic DNA from patient tumor tissue. The amplification of the KRAS gene was performed using a master mix comprising template DNA, specific primers, a fluorescence probe, DNA polymerase, deoxyribonucleotide triphosphates (dNTPs), and a reaction buffer. The thermal cycling process included denaturation, annealing, and elongation, with real-time detection of amplification products achieved through the measurement of fluorescence intensity during PCR cycles. Data analysis was based on the interpretation of amplification curves, where a significant increase in fluorescence signal indicated the presence of mutations in the KRAS gene.

Determination of Mismatch Repair (MMR) Status

The mismatch repair (MMR) status was determined using immunohistochemistry (IHC) to evaluate the expression of key proteins (MLH1, MSH2, MSH6, and PMS2) from Vantage Biosciences (London, UK; catalog numbers 285M-14, 286M-14, 287M-14, and 288M-14). Paraffin-fixed tumor tissue sections underwent a series of steps, including deparaffinization, rehydration, and antigen retrieval. Subsequently, endogenous peroxidase blocking was performed before incubation with primary antibodies specific to MMR proteins. The detection of primary antibodies was carried out using secondary antibodies conjugated with horseradish peroxidase (HRP), followed by visualization with diaminobenzidine (DAB) substrate and nuclear counterstaining with hematoxylin. The MMR status of the tumor was classified based on the expression patterns of MMR proteins observed under a light microscope. Loss of expression of one or more proteins indicated a deficient MMR (dMMR) status, whereas intact expression of all proteins indicated a proficient MMR (pMMR) status.

Data were organized and analyzed using SPSS version 25.0 for windows. Continuous variables were presented as mean ± standard deviation or median with interquartile range, while categorical variables were expressed as frequencies.

Results

Data Overview and Patient Characteristics

Initially, data were collected from 55 patients; however, the analysis focused on 23 patients with complete KRAS mutation data, which is crucial for examining the relationship between KRAS mutations and MMR status.

The sex distribution revealed a significant disparity, with females comprising 65.2% of the sample and males 34.8%. This imbalance suggests potential sex-related influences on the study's outcomes, which should be considered in future analyses. The age distribution indicated that the majority of patients (37.0%) were within

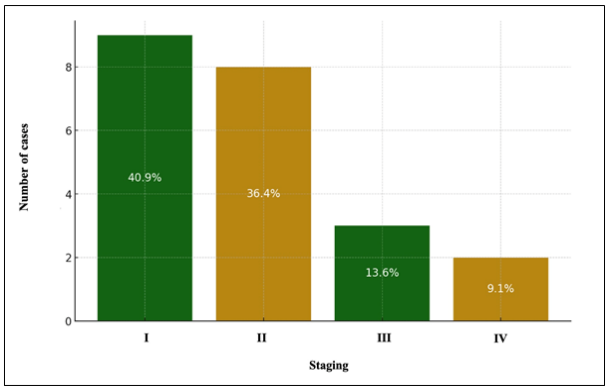

Figure 1. Distribution of Cases by Staging

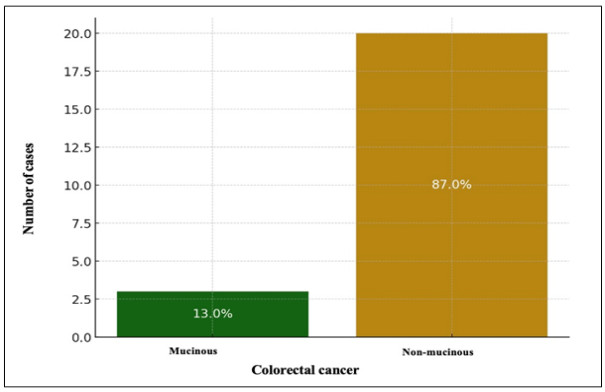

Figure 2. Distribution of Mucinous and Non-Mucinous Colorectal Cancer Cases

the 50–59 age group, consistent with the higher prevalence of CRC in this demographic. Additionally, 29.6% of patients were aged 60–69, indicating that CRC remains a significant concern in older populations. The frequency of patients decreased in the 70–79 (18.5%) and over 80 (3.7%) age groups, which may suggest either a lower disease prevalence at advanced ages or external factors such as increased mortality affecting patient numbers.

KRAS Mutation Frequency and Cancer Staging

The distribution of cancer stages among patients showed notable variation, with a substantial proportion diagnosed at an early stage. Specifically, 40.9% were in Stage I, suggesting that nearly half of the study population received early diagnoses (Figure 1). Another 36.4% were in Stage II, reinforcing the effectiveness of current early detection efforts. However, only 13.6% of patients were diagnosed at Stage III, and 9.1% at Stage IV, the most advanced stage of the disease.

Tumor Characteristics and Location

Analysis of tumor histology showed that 87% of patients had non-mucinous colorectal cancer, while 13% had mucinous tumors (Figure 2). Regarding tumor burden, 90.5% of patients had single tumors, while 9.5% had multiple tumors, indicating either metastasis or multiple primary malignancies.

Tumor location also played a critical role in disease presentation and management. The study found that 61.9% of patients had left-sided colon tumors (descending colon), while 38.1% had right-sided tumors (ascending colon) (Figure 3).

Association Between KRAS Mutations and MMR Status

Data analysis revealed that 91.3% of patients with KRAS mutations had pMMR status, while only 8.7% had dMMR (Figure 4).

Discussion

This study found that 91.3% of CRC patients had pMMR status, while only 8.7% had dMMR, with KRAS mutations predominantly occurring in pMMR cases. Among the patients, 77.3% were diagnosed at early stages (I and II), while 22.7% were in advanced stages (III and IV). Additionally, 61.9% of tumors were located in the left colon, and 87% were non-mucinous.

Previous research suggests that the presence of KRAS mutations, particularly in conjunction with pMMR status, may influence the effectiveness of certain cancer treatments and the overall prognosis of patients [1, 21]. The high proportion of early-stage diagnoses (77.3%) reflects the success of screening programs; however, the presence of advanced-stage cases underscores the need for continued efforts in early detection and timely intervention. Delays in diagnosis and disparities in healthcare access remain critical factors in advanced-stage presentations [6]. The detection of patients at later stages (22.7%), however, highlights persistent gaps in early diagnosis, potentially due to disparities in healthcare access or delays in symptom recognition [22]. In this study, mucinous cancers were found to be less common. Mucinous cancers are often associated with more aggressive biological behavior and variable responses to chemotherapy. Although less common, mucinous tumors may require more individualized treatment approaches due to their distinct molecular characteristics and potential resistance to standard therapies.

In this study, the predominance of single tumors suggests that most cases presented with localized disease, which is generally associated with better prognoses and higher responsiveness to localized treatments such as surgery or radiation. In contrast, patients with multiple

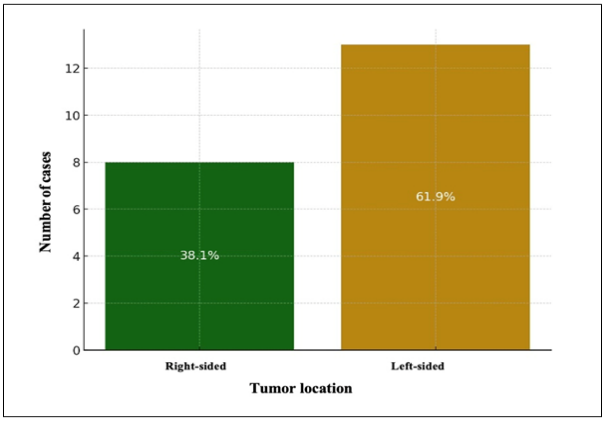

Figure 3. Tumor Location Distribution

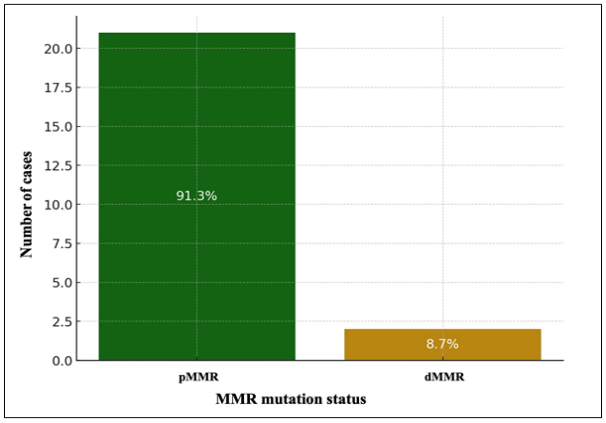

Figure 4. Status Distribution Patients with KRAS Mutations had MMR Mutation

tumors are more likely to require aggressive therapeutic strategies, including systemic chemotherapy or targeted therapies

In examining the relationship between KRAS mutations and MMR status, we found that 91.3% of patients with KRAS mutations had pMMR, while only 8.7% had dMMR. This suggests that KRAS mutations are more prevalent in cases where DNA repair mechanisms, though not flawless, remain largely intact [23]. Conversely, the lower prevalence of KRAS mutations in dMMR patients may indicate that CRC in this subgroup primarily progresses through microsatellite instability rather than KRAS-driven pathways [7].

These findings have significant clinical implications. The association between KRAS mutations and pMMR status suggests that pMMR patients may benefit from different therapeutic approaches than those with dMMR, who are more likely to respond to immune-based therapies [9]. Further research is needed to explore the underlying molecular pathways and refine treatment strategies that incorporate both KRAS mutation status and MMR proficiency [24].

This study has several limitations. First, the sample had an uneven distribution of demographic and clinical variables, with females comprising the majority (65.2%). This imbalance may influence the interpretation of the relationship between KRAS mutations and MMR status. Additionally, the proportion of patients with advanced-stage cancer (stages III and IV) was relatively low (22.7%), which may limit our understanding of the role of KRAS mutations in advanced disease. Second, this study focused solely on KRAS mutations and MMR status, without considering other molecular factors that contribute to CRC development, such as BRAF mutations or the expression of proteins involved in alternative signaling pathways. Third, the study was conducted at a single research site Wahidin Sudirohusodo Hospital and its affiliated hospitals in Makassar. As a result, the findings may not be generalizable to the broader CRC population in Indonesia.

In conclusion, KRAS mutations are more frequently associated with pMMR in CRC, indicating distinct molecular pathways. Personalized therapeutic approaches should consider both KRAS mutation status and MMR proficiency to optimize treatment outcomes. However, due to the limited number of dMMR patients in this study, further research with a larger sample size is necessary to confirm these findings.

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Statement of Transparency and Principals:

- Author declares no conflict of interest
- Study was approved by Research Ethic Committee of author affiliated Institute.
- Study's data is available upon a reasonable request.
- All authors have contributed to implementation of this research.

References

1. Mármol I, Sánchez-de-Diego C, Pradilla Dieste A, Cerrada E, Rodriguez Yoldi MJ. Colorectal Carcinoma: A General Overview and Future Perspectives in Colorectal Cancer. International Journal of Molecular Sciences. 2017 01 19;18(1):197. https://doi.org/10.3390/ijms18010197
2. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2024;74(3):229-263. https://doi.org/10.3322/caac.21834
3. Andinata B, Bachtiar A, Oktamianti P, Partahi JR, Dini MSA. A Comparison of Cancer Incidences Between Dharmais Cancer Hospital and GLOBOCAN 2020: A Descriptive Study of Top 10 Cancer Incidences. Indonesian Journal of Cancer. 2023 06 21;17(2):119-122. https://doi.org/10.33371/ijoc.v17i2.982
4. Prihantono N, Rusli R, Christeven R, Faruk M. Cancer Incidence and Mortality in a Tertiary Hospital in Indonesia: An 18-Year Data Review. Ethiopian Journal of Health Sciences. 2023 05;33(3):515-522. https://doi.org/10.4314/ejhs.v33i3.15
5. Jinesh GG, Sambandam V, Vijayaraghavan S, Balaji K, Mukherjee S. Molecular genetics and cellular events of K-Ras-driven tumorigenesis. Oncogene. 2018 02 15;37(7):839-846. https://doi.org/10.1038/onc.2017.377
6. Dohrn N, Klein MF. Colorectal cancer: current management and future perspectives. The British Journal of Surgery. 2023 09 06;110(10):1256-1259. https://doi.org/10.1093/bjs/znad095
7. Zhu G, Pei L, Xia H, Tang Q, Bi F. Role of oncogenic KRAS in the prognosis, diagnosis and treatment of colorectal cancer. Molecular cancer. 2021 Nov 06;20(1). https://doi.org/10.1186/s12943-021-01441-4
8. Andrade F, German-Cortés J, Montero S, Carcavilla P, Baranda-Martínez-Abascal D, Moltó-Abad M, Seras-Franzoso J, Díaz-Riascos ZV, Rafael D, Abasolo I. The Nanotechnology-Based Approaches against Kirsten Rat Sarcoma-Mutated Cancers. Pharmaceutics. 2023 06 08;15(6):1686. https://doi.org/10.3390/pharmaceutics15061686
9. Bupathi M, Wu C. Biomarkers for immune therapy in colorectal cancer: mismatch-repair deficiency and others. Journal of Gastrointestinal Oncology. 2016 Oct;7(5):713-720. https://doi.org/10.21037/jgo.2016.07.03
10. Giaretti W, Venesio T, Sciutto A, Prevosto C, Geido E, Risio M. Near-diploid and near-triploid human sporadic colorectal adenocarcinomas differ for KRAS2 and TP53 mutational status. Genes, Chromosomes & Cancer. 2003 06;37(2):207-213. https://doi.org/10.1002/gcc.10203
11. Nguyen LH, Goel A, Chung DC. Pathways of Colorectal Carcinogenesis. Gastroenterology. 2020 01;158(2):291-302. https://doi.org/10.1053/j.gastro.2019.08.059
12. Svoboda M, Sana J, Fabian P, Kocakova I, Gombosova J, Nekvindova J, Radova L, Vyzula R, Slaby O. MicroRNA expression profile associated with response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer patients. Radiation Oncology (London, England). 2012 Nov 20;7:195. https://doi.org/10.1186/1748-717X-7-195
13. Li H, Zhang Y, Cai J, Bian H. MicroRNA-451 inhibits growth of human colorectal carcinoma cells via downregulation of Pi3k/Akt pathway. Asian Pacific journal of cancer prevention: APJCP. 2013;14(6):3631-3634. https://doi.org/10.7314/apjcp.2013.14.6.3631
14. Lou X, Qi X, Zhang Y, Long H, Yang J. Decreased expression

of microRNA-625 is associated with tumor metastasis and poor prognosis in patients with colorectal cancer. Journal of Surgical Oncology. 2013 09;108(4):230-235. https://doi.org/10.1002/jso.23380
15. Rasool M, Carracedo A, Sibiany A, Al-Sayes F, Karim S, Haque A, Natesan Pushparaj P, Asif M, Achakzai NM. Discovery of a novel and a rare Kristen rat sarcoma viral oncogene homolog (KRAS) gene mutation in colorectal cancer patients. Bioengineered. 2021 Dec;12(1):5099-5109. https://doi.org/10.1080/21655979.2021.1960715
16. Kim T, Hwang SW, Kim KO, Cha JM, Joo Y, Cho Y. The Prognostic Utilities of DNA Mismatch Repair Status and KRAS and BRAF Mutation in Korean Colorectal Cancer Patients: The KASID Multicenter Study. Oncology. 2023;101(1):49-58. https://doi.org/10.1159/000527285
17. Lian S, Tan L, Liu X, Yang L, Li N, Feng Q, Wang P, et al. KRAS, NRAS, BRAF signatures, and MMR status in colorectal cancer patients in North China. Medicine. 2023 03 03;102(9):e33115. https://doi.org/10.1097/MD.0000000000033115
18. Cionca FL, Dobre M, Dobrea CM, Iosif CI, Comănescu MV, Ardeleanu CM. Mutational status of KRAS and MMR genes in a series of colorectal carcinoma cases. Romanian Journal of Morphology and Embryology = Revue Roumaine De Morphologie Et Embryologie. 2018;59(1):121-129. https://doi.org/kkkk
19. Mamonto L, Nelwan BJ, Sungowati NK, Miskad UA, Cangara MH, Zainuddin AA. Association of chemokine (CXC motif) receptor 4 expression with lymphovascular invasion and lymph node metastasis of invasive breast cancer. Breast Disease. 2022;41(1):447-453. https://doi.org/10.3233/BD-229003
20. Cahyaningtyas C, Muslich LT, Madjid B, Sultan AR, Hamid F, Hatta M. Factors associated with Leptospira serodiagnosis in febrile patients at public health centers in Makassar, Indonesia: a cross-sectional study. The Pan African Medical Journal. 2024;49:113. https://doi.org/10.11604/pamj.2024.49.113.45645
21. Mattiuzzi C, Sanchis-Gomar F, Lippi G. Concise update on colorectal cancer epidemiology. Annals of Translational Medicine. 2019 Nov;7(21):609. https://doi.org/10.21037/atm.2019.07.91
22. Lewandowska A, Rudzki G, Lewandowski T, Stryjkowska-Góra A, Rudzki S. Risk Factors for the Diagnosis of Colorectal Cancer. Cancer Control: Journal of the Moffitt Cancer Center. 2022;29:10732748211056692. https://doi.org/10.1177/10732748211056692
23. Sinicrope FA, Shi Q, Smyrk TC, Thibodeau SN, Dienstmann R, Guinney J, Bot BM, Tejpar S, Delorenzi M, Goldberg RM, Mahoney M, Sargent DJ, Alberts SR. Molecular markers identify subtypes of stage III colon cancer associated with patient outcomes. Gastroenterology. 2015 01;148(1):88-99. https://doi.org/10.1053/j.gastro.2014.09.041
24. Li W, Zhi W, Zou S, Qiu T, Ling Y, Shan L, Shi S, Ying J. Distinct Clinicopathological Patterns of Mismatch Repair Status in Colorectal Cancer Stratified by KRAS Mutations. PloS One. 2015;10(6):e0128202. https://doi.org/10.1371/journal.pone.0128202