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Original Article

Molecular Characteristics of Colorectal Cancer: Association of Kirsten Rat Sarcoma Viral Oncogene Homologue, Neuroblastoma RAS Viral Oncogene Homologue, and B-Raf Proto-oncogene, Serine/Threonine Kinase Mutations with Tumor Location in a Bangladeshi Cohort

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Abstract

Introduction: Colorectal cancer (CRC) is a common malignancy with significant genetic heterogeneity. Mutations in proto-oncogenes such as Kirsten rat sarcoma viral oncogene homologue (KRAS), Neuroblastoma RAS viral oncogene homolog (NRAS), and B-Raf proto-oncogene, serine/threonine kinase (BRAF) play a pivotal role in CRC development impacting prognosis and treatment. This study aims to correlate mutations in these genes with tumor localization in both primary and metastatic Colorectal Carcinoma (CRC).

Methods: This prospective cross-sectional study was conducted between July 2023 and June 2024 at BSMMU. A total of 30 CRC patients, confirmed via histopathology, were included. Purposive sampling was used to select patients. Tumor tissue samples were collected and analyzed for KRAS, NRAS, and BRAF mutations using DNA isolation, Polymerase Chain Reaction (PCR) amplification, and sequencing techniques.

Results: Among the 30 patients, the majority were male (66.7%) with a mean age of 50.4 years. KRAS mutations were found in 5 patients (16.7%), while no mutations in NRAS or BRAF were detected. Rectal cancer was the most frequent tumor location (36.7%), followed by hepatic and splenic flexure (16.7% each). No significant correlation was observed between KRAS mutations and tumor localization.

Conclusions: There was no statistically significant correlation between KRAS, NRAS, and BRAF mutations and tumor localization in the BSMMU CRC patient cohort. The study highlights the need for larger sample sizes to better understand the genetic landscape of CRC in Bangladesh. Small sample size may limit the ability to detect significant associations. Further large-scale studies could offer more conclusive insights.

Keywords: Colorectal cancer, KRAS, NRAS, BRAF, DNA isolation, PCR amplification

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Introduction

Colorectal cancer (CRC) is one of the most common malignancies worldwide, accounting for over 10% of cancer cases, and ranks as the second leading cause of cancer-related mortality globally.[1] Early-stage CRC has favorable outcomes with high survival rates when detected and treated promptly. However, approximately 10% of early-stage CRC patients experience poor outcomes, highlighting the complexity of the disease.[2] The understanding of CRC pathogenesis has evolved considerably and it is now known that genetic alterations play a pivotal role in the development and progression of this disease. Kirsten rat sarcoma viral

oncogene homologue (KRAS), Neuroblastoma RAS viral oncogene homolog (NRAS), and B-Raf proto-oncogene, serine/threonine kinase (BRAF) are key oncogenes involved in colorectal carcinogenesis. Mutations in these genes can disrupt cell growth and survival pathways, notably the RAS-RAF-MEK-ERK pathway, contributing to tumor development and progression.[3] KRAS mutations are found in approximately 40% of CRC cases, particularly affecting codons 12 and 13, and are often associated with poor prognosis and resistance to therapies targeting the epidermal growth factor receptor(EGFR), such as cetuximab and panitumumab.[4,5] Similarly, BRAF mutations, which occur in about 10% of CRC cases, especially in the V600E codon, are linked to more aggressive tumor behavior and poor survival rates.[6,7]

While the genetics and scope of CRC have been extensively studied in Western populations, there is limited data on the prevalence of KRAS, NRAS, and BRAF mutations and their relationship with tumor localization in Bangladeshi patients. Understanding these mutations in the Bangladeshi population is critical for optimizing treatment strategies, particularly in light of the increasing use of targeted therapies. This study aims to investigate the correlation between KRAS, NRAS, and BRAF mutations and the anatomical location of primary and metastatic colorectal tumors in a cohort of Bangladeshi patients treated at Bangladesh Medical University (BMU).

Methods

This prospective cross-sectional study was conducted at the department of colorectal surgery, BSMMU, between July 2023 and June 2024. A total of 30 patients with histopathologically confirmed CRC were recruited. Inclusion criteria encompassed patients over the age of 18, diagnosed with adenocarcinoma of the colon or rectum, and those who provided informed consent. Patients with obstructed or perforated CRC or those with recurrent disease were excluded.

Tissue samples were collected from surgical specimens and processed for genetic analysis. DNA was extracted using the Gene JET™ DNA extraction kit and subjected to polymerase chain reaction (PCR) amplification. Specific primers were used to amplify regions of the KRAS, NRAS, and BRAF genes. Following amplification, PCR products were sequenced, and the presence of mutations was confirmed using sequence analysis software. The frequency of mutations was analyzed, and their correlation with tumor localization was assessed.

Results

A total of 30 patients with histopathologically confirmed colorectal cancer were included in the study. The mean age was 50.4±10.9 years (range: 25–72 years), with the majority of patients (70.0%) belonging to the 41–60-year age group. Male patients predominated, accounting for 66.7% (n=20), resulting in a male-to-female ratio of 2:1. The demographic characteristics are summarized in Table I.

Table I: Patient demographics, tumor location, and mutation distribution (n=30).

Variables	Categories	Number	Percentage(%)
Age(in years)	≤40	6	20.0
	41-60	21	70.0
	>60	3	10.0
	Mean ± SD	50.4±10.9	
	Range(min-max)	25-72	
	Male	20	66.7
Sex	Female	10	33.3
	Rectum	11	36.7
	Hepatic flexure	5	16.7
	Splenic flexure	5	16.7
Tumor location	Ascending colon	4	13.3
	Sigmoid colon	2	6.7
	Descending colon	2	6.7
	Caecum	1	3.3
Gene mutation	KRAS	5	16.7
	BRAF	0	0.0
	NRAS	0	0.0
	No mutation	25	83.3

Table II : Correlation between tumor location and mutation (n=30).

Variables	Category	Metastatic, n=5) (%)	Non-metastatic, (n=25) (%)	P value	KRAS mutated, (n=5)(%)	KRAS not mutated, (n=25) (%)	P value
Tumor location	Rectum	2 (40.0%)	9 (36.0%)	0.619	0(0.0%)	11 (44.0%)	0.082
	Hepatic flexure	0 (0.0%)	5 (20.0%)	0.373	1 (20.0%)	4 (16.0%)	0.627
	Splenic flexure	0 (0.0%)	5 (20.0%)	0.373	1 (20.0%)	4 (16.0%)	0.627
	Ascending colon	1 (20.0%)	3 (12.0%)	0.538	0 (0.0%)	4 (16.0%)	0.462
	Sigmoid colon	0 (0.0%)	2 (8.0%)	0.690	1 (20.0%)	1 (4.0%)	0.310
	Descending colon	1 (20.0%)	1 (4.0%)	0.310	1 (20.0%)	1 (4.0%)	0.310
	Caecum	1 (20.0%)	0 (0.0%)	0.167	1 (20.0%)	0 (0.0%)	0.167
KRAS mutation	Yes	2 (40.0%)	3 (12.0%)	0.183			
	No	3(60.0%)	22 (88.0%)				

*P-values are calculated using chi-square tests; ns= not significant.

Regarding tumor distribution, the rectum was the most common primary site, representing 36.7% (n=11) of cases. Tumors located at the hepatic flexure and splenic flexure each accounted for 16.7% (n=5), followed by the ascending colon (13.3%, n=4), sigmoid colon (6.7%, n=2), descending colon (6.7%, n=2), and caecum (3.3%, n=1). Overall, left-sided colorectal tumors constituted the majority of cases, reflecting the predominance of distal colorectal malignancies observed in many South Asian populations.

Molecular analysis demonstrated KRAS mutations in 5 patients (16.7%), whereas no NRAS or BRAF mutations were detected. Consequently, 83.3% (n=25) of patients were classified as wild-type for all three genes investigated. The low prevalence of KRAS mutations and complete absence of NRAS and BRAF alterations suggest a distinct molecular profile within this cohort, although interpretation should be undertaken cautiously due to the limited sample size.

Correlation analysis between tumor location and KRAS mutation status revealed no statistically significant association (all p>0.05). Among KRAS-mutated tumors, the hepatic flexure, splenic flexure, sigmoid colon, descending colon, and caecum each contributed one case (20.0%), while no KRAS mutation was identified in rectal or ascending colon cancers. Similarly, no significant relationship was observed between metastatic status and tumor location. Two of the five KRAS-mutated patients (40.0%) had metastatic disease compared with 12.0% among KRAS wild-type patients; however, this difference did not reach statistical significance (p=0.183). These findings suggest that tumor localization alone may not reliably predict KRAS mutation status in Bangladeshi patients with colorectal cancer.

Discussion

The present study evaluated the distribution of KRAS, NRAS, and BRAF mutations and their association with tumor localization among Bangladeshi patients with colorectal cancer. KRAS mutations were identified in 16.7% of cases, whereas no NRAS or BRAF mutations were detected. Furthermore, no statistically significant relationship was observed between molecular alterations and primary tumor location. These findings contribute to the limited body of molecular epidemiological data available from Bangladesh and provide preliminary insights into the genetic landscape of colorectal cancer within the region.

KRAS is the most frequently mutated oncogene in colorectal cancer and plays a central role in activation of the RAS–RAF–MEK–ERK signaling cascade, promoting cellular proliferation, survival, invasion, and metastatic progression.[3-5] Large international studies have reported KRAS mutation frequencies ranging from 30% to 45%, substantially higher than the 16.7% observed in our cohort.[5,13,14] Similar variation has been reported across Asian populations, where mutation prevalence ranges from 15% to 40%, influenced by ethnicity, tumor stage, sample selection, and

molecular testing methodology.[7] The lower mutation rate observed in this study may therefore reflect both population-specific biological characteristics and the relatively small sample size.

Tumor sidedness has emerged as an important molecular and prognostic determinant in colorectal cancer. Right-sided tumors are frequently associated with microsatellite instability (MSI), CpG island methylator phenotype (CIMP), and BRAF mutations, whereas left-sided tumors more commonly demonstrate chromosomal instability and EGFR pathway activation.[15,16] Several investigators have reported higher frequencies of KRAS mutations in proximal colon cancers compared with distal colorectal tumors.[9,10] However, our study did not identify any significant association between KRAS mutation and anatomical tumor location. Similar findings have been reported by several Asian cohorts, suggesting that the relationship between tumor sidedness and molecular alterations may vary across populations.[7,17]

The absence of NRAS mutations in this cohort is noteworthy. NRAS mutations are generally uncommon, occurring in approximately 2–5% of colorectal cancers, and are most frequently detected in metastatic disease.[18] Although rare, these mutations are clinically relevant because they predict resistance to anti-EGFR monoclonal antibodies, similar to KRAS alterations. The absence of NRAS mutations in the current study is likely attributable to limited sample size rather than true absence within the Bangladeshi population.

Likewise, no BRAF mutation was detected. In Western populations, BRAF V600E mutations occur in approximately 8–15% of colorectal cancers and are associated with right-sided tumor location, advanced stage, poor differentiation, microsatellite instability, and inferior survival outcomes.[6,12,19] The absence of BRAF mutations in our series contrasts with these reports but is consistent with observations from several Asian studies where BRAF mutation frequencies tend to be considerably lower.[7,20] These geographic differences suggest potential ethnic and molecular heterogeneity in colorectal carcinogenesis.

From a therapeutic perspective, molecular characterization of colorectal cancer has become indispensable in the era of precision oncology. Current international guidelines recommend routine testing for KRAS, NRAS, and BRAF mutations in patients with metastatic colorectal cancer to guide selection of targeted therapies.[21] Patients harboring KRAS or NRAS mutations derive little benefit from anti-EGFR agents such as cetuximab and panitumumab, while BRAF-mutated tumors may benefit from combination targeted approaches incorporating BRAF inhibitors and EGFR blockade.[11,21] Therefore, implementation of standardized molecular testing should become an integral component of colorectal cancer management in Bangladesh.

The principal limitation of this study is the relatively small sample size, which reduces statistical power and increases the risk of type II error. Additionally, the single-center design may limit generalizability. Future multicenter studies incorporating larger cohorts, next-generation sequencing platforms, MSI/MMR status, HER2 amplification, and additional molecular biomarkers are required to establish a comprehensive genomic profile of colorectal cancer in Bangladesh. Such efforts may facilitate development of population-specific treatment algorithms and contribute to precision oncology initiatives within the country.

Conclusion

This study demonstrated that KRAS mutations were present in a subset of CRC patients treated at BSMMU, but no significant correlation was found between KRAS mutations and tumor localization. The absence of NRAS and BRAF mutations in this cohort highlights the need for larger studies to explore the genetic landscape of CRC in Bangladesh. Molecular testing for these mutations is crucial for guiding treatment decisions, particularly in the context of targeted therapies.

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Conflict of interest

None declared

Ethical approval

The study was approved by the Institutional Ethics Committee

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