Biomedical Research and Therapy 2024, 11(9):6723-6729

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Report

Mutational variants of KRAS gene versus Wild-type KRAS in the survival outcomes of Vietnamese colon cancer stage II-III

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ABSTRACT

Background: Colon cancer is one of the most common cancers in Viet Nam and globally. The *KRAS* gene (Kirsten rat sarcoma) is an oncogene showing a high mutation rate in colon cancer, affecting 30% to 40% of patients. The mutated *KRAS* gene, which keeps the MAPK signaling pathway permanently active, is considered a negative factor in the survival of colon cancer patients. Recently, several studies have been conducted to evaluate the specific prognoses related to distinct *KRAS* mutations, but the results were controversial. Mutations in different KRAS codons may impact colon cancer treatment models and prognoses. Therefore, the impacts of codon-specific *KRAS* mutations on survival require further clarification. This study aims to determine the associations between codon-specific *KRAS* mutations and survival in Vietnamese patients at stages II - III.

Methods: A descriptive design was applied and included 158 colon cancer patients at stages II-III at Bach Mai Hospital from January 2016 to August 2020. Testing for KRAS mutations was performed with formalin-fixed, paraffin-embedded tissue, and *KRAS* mutations were detected with the KRAS XL StripAssay (ViennaLab, Austria). **Results**: Among 158 patients, 71 (44.9%) exhibited mutated *KRAS* genes. The most frequent mutated KRAS variant was p.Gly12Asp (G12D) at 35.2%; the proportion of KRAS G13D (p.Gly13Asp) was 15.5%. The 3-year DFS of the wild-type KRAS group was 60.3%, while that of the mutated KRAS Exon 2 group was 55.2%. The worse prognosis for DFS was observed in patients with KRAS codon 12 mutations (3-year DFS: 52.9%). Among them, patients with codon-specific KRAS mutations named p.Gly12Asp (G12D) and p.Gly12Val (G12V) experienced better prognoses (59.1% and 63.6%, respectively). However, the differences were not statistically significant (P > 0.05). **Conclusion**: KRAS p.Gly12Asp (G12D) was the most common type among mutated *KRAS* genes. Codon-specific KRAS mutation was not a prognostic factor for DFS and OS in colon cancer stages II-III. The results support the current clinical practice that determining specific codon *KRAS* gene status is not conventional testing for resected colon cancer stages II-III.

Key words: Colon cancer, disease-free survival, G12D, KRAS gene, overall survival, stage II-III

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History

- Received: Jul 01, 2024
- Accepted: Sep 24, 2024
- Published Online: Sep 30, 2024

DOI : 10.15419/bmrat.v11i9.916

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INTRODUCTION

Colon cancer (CC) is one of the most common cancers in Vietnam and globally [1]. Many years of biological feature discovery attempts have provided opportunities for personalized treatment, thereby supporting the reduction of mortality from CC. The K*RAS* gene (Kirsten rat sarcoma) is an oncogene known for its high mutation rate in CC, affecting 30% to 40% of patients [2,3]. The MAPK signaling pathway plays a significant role in the proliferation of cancer cells. The mutated K*RAS* gene, which keeps the aforementioned pathway permanently active, is considered a negative factor for survival in CC patients. K*RAS* mutations in exon 2 at codons 12 and 13 are predominant. Minor rates of mutations at codons 59 and 61 in exon 3 have been reported, while mutations at codons 117 and 146 are rarely detected. Additionally, many variants of K*RAS* mutations have been identified. Among them, the most frequent KRAS types are G12D (p.Gly12Asp), G12V (p.Gly12Val), G12C (p.Gly12Cys), and G13D (p.Gly13Asp) [4–6]. KRAS G12D is the most common KRAS mutation detected in carcinomas [7]. Recently, several studies have evaluated the specific prognoses related to individual KRAS mutations, but the results have been controversial [8,9]. Therefore, the impacts of codon-specific KRAS mutations on survival require further clarification.

CC cases show an increasing trend in Vietnam, and most patients are diagnosed at pathological stages II or III [10]. However, there are only a few studies on KRAS status in CC patients, and the associations between codon-specific KRAS mutations and survival have not been elucidated [3,11,12]. Individual treatment based on a patient's genetic information plays a significant role in patient management and decision-making. Therefore, this study aims to determine the associations between codon-specific KRAS mutations and survival in Vietnamese patients at stages II-III.

Cite this article : Cuong H M, Loi N T, Ngoc T B, Thang V H. **Mutational variants of KRAS gene versus Wild-type KRAS in the survival outcomes of Vietnamese colon cancer stage II-III**. *Biomed. Res. Ther.* 2024; 11(9):6723-6729.

METHODS

Data in this study covered all patients (n = 158) with colon cancer diagnosed at pathological stages II - III who were admitted to Bach Mai Hospital (Hanoi, Vietnam) from January 2016 to August 2020. The inclusion criteria include pathological stages II-III colon cancer according to the 8^{th} edition of the AJCC staging system, receiving radical treatment, testing for the KRAS status with determination of mutated sites, and patients' documents being fully accessible. The exclusion criteria include the diagnosis of a second cancer and the inability to answer the research questions due to illness.

Study Design

A descriptive design was applied. The significant research objects were the correlations of single KRAS mutations with disease-free survival (DFS) and overall survival (OS). The observation period began on the date of receiving radical surgery.

Mutation Analysis

Testing for KRAS mutations was performed with formalin-fixed, paraffin-embedded (FFPE) tissue. Paraffin in FFPE tissue slides was eliminated by using an FFPE deparaffinization solution (MERCK, Germany). DNA was then extracted from tissue samples with the aid of a PureLink™ Genomic DNA Mini Kit (Invitrogen, USA). KRAS mutations were detected with the KRAS XL StripAssay (ViennaLab, Austria) in a five-step procedure that included amplification, hybridization, stringent wash, and color development. The strips were analyzed to determine the mutational variants of the KRAS gene at codons 12, 13, 59, 60, 61, 117, and 146. [13,14]

Statistical Analyses

Statistical analyses were performed using SPSS 21.0 software. The Kaplan-Meier method was used to calculate the survival rate, and the log-rank test was applied to compare the survival rates of two groups. A P-value of less than 0.05 was considered statistically significant.

Ethics Declarations

The study was permitted by the Ethics Committee of Hanoi Medical University (approval number: NCS28/HMU-IRB). The patients consented to participate in the study.

RESULTS

Patients' Characteristics

A total of 158 patients were recruited for this study. Among them, 86/158 (54.4%) were males, and those aged 60-69 accounted for the highest proportion at 36.1%. The predominant histological type is adenocarcinoma, constituting 86.7% (137/158) of all cases, while other types were identified in 21 cases (13.3% of patients). The T4 stage (including T4a and T4b) was confirmed in 91 patients (57.5%); Stage III tumors were identified at a higher rate than Stage II tumors (52.5% vs. 44.9%). The KRAS-mutated rate was 44.9% (71/158) compared with 55.1% (87/158) of the KRAS wild-type rate (**Table 1**).

Mutated Types of KRAS Gene in Colon Cancer Stage II–III

Among the 71 patients diagnosed with a mutated KRAS gene, the most frequent mutated KRAS variant was p.Gly12Asp (G12D) at 35.2%. The proportion of KRAS G13D (p.Gly13Asp) was 15.5%. All the mutated KRAS types of p.Gly>Val and p.Gly>Ser were identified at codon 12 (G12V and G12S, respectively), with rates of 15.5% and 8.5%. The other types, which accounted for 12.7% (9 out of 71 cases), include mutated KRAS variants: p.Ala146Val (A146V); p.Ala59Gly (A59G); p.Gly12Ala (G12A); p.Gly12Arg (G12R); p.Gly13Arg (G13R), p.Gly12Cys (G12C), and p.Lys117Asn (K117N) (**Figure 1**).

Association of Codon-Specific KRAS Mutations with Survival in Colon Cancer Stage II–III

The median follow-up duration was 40.0 months (min, 12 months; max, 78 months) (not shown in any table). Patients with KRAS mutations commonly experienced lower rates of 3-year DFS and 4-year OS, but the differences were minor and not statistically significant. The 3-year DFS of the wild-type KRAS group was 60.3%, while that of the mutated KRAS exon 2 group was 55.2%; however, the difference was not statistically significant (P = 0.592). Similarly, the 4-year OS of the mutated KRAS exon 2 and wild-type groups were 69.5% and 69.9%, respectively (P = 0.933). The worse prognosis for DFS was shown in patients with KRAS codon 12 mutations (3-year DFS; 52.9%). Among them, patients with codon-specific KRAS mutations p.Gly12Asp (G12D) and p.Gly12Val (G12V) had better prognoses (59.1% and 63.6%, respectively).

Table 1: Patients' characteristics

Characteristic		Total (n = 158)	Percentage (%)
Age			
	< 40 ys	17	10.8
	40 - 49 ys	28	17.7
	50 – 59 ys	42	26.6
	60 – 69 ys	57	36.1
	≥ 70 ys	14	8.9
Sex			
	Male	86	54.4
	Female	72	45.6
Histological types			
	Adenocarcinoma	137	86.7
	Mucinous Adenocarcinoma	18	11.4
	Others*	3	1.9
Tumor sites			
	Right	76	48.1
	Left	82	51.9
Tumor invasion			
	pT3	67	42.4
	pT4	91	57.5
Lymph node stage			
	pN0	75	47.5
	pN1	59	37.3
	pN2	24	15.2
pTNM stage			
	II	75	47.5
	III	83	52.5
KRAS status			
	Mutated	71	44.9
	Wild - type	87	55.1

* Signet ring carcinoma and Micropapillary carcinoma

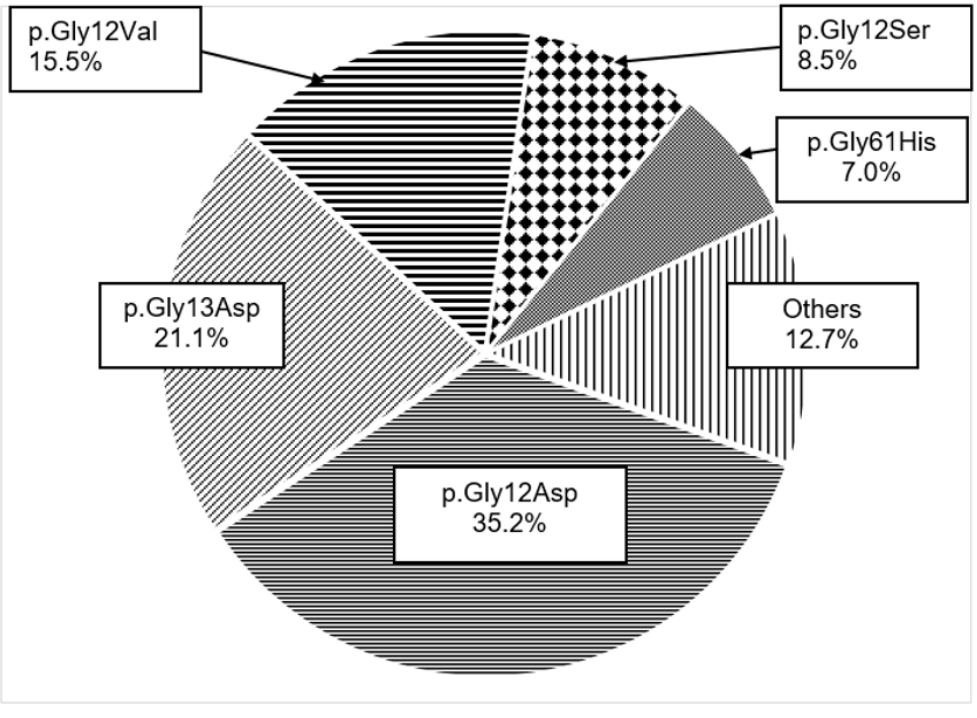

Figure 1: **Mutational variants of KRAS gene**.

Table 2: **Associations between codon-specific KRAS mutations and survival in specified groups**

Specified group	n	3-year DFS	p-value*	4-year OS	p-value*
Mutated KRAS Exon 2 (Codon 12, 13)	63	55.2%	0.592	69.5%	0.933
Mutated KRAS codon 12	47	52.9%	0.448	69.7%	0.937
Mutated KRAS codon 13	16	61.9%	0.844	70.7%	0.708
Mutated type of p.Gly>Asp (Codon 12, 13)	40	59.2%	0.989	69.1%	0.873
Mutated type of p.Gly12Asp (G12D)	25	59.1%	0.983	71.6%	0.896
Mutated type of p.Gly13Asp (G13D)	15	59.3%	0.989	69.9%	0.897
Mutated type of Gly12Val (G12V)	11	63.6%	0.881	72.7%	0.962
KRAS wild-type	87	60.3%		69.9%	
* compared with KRAS wild-type					

DISCUSSION

The Raf/Ras/MAPK pathway is the downstream signaling pathway of EGFR; more than one-half of colorectal cancers are indicated with *EGFR* overexpression. Although overexpression of *EGFR* is not predictive of response to anti-EGFR therapy[15], *KRAS* gene status plays a vital role in selecting candidates for anti-EGFR treatment in advanced colorectal cancer[16]. The impacts of *KRAS* gene status on prognosis and treatment choices in CC stage II-III are unstable; some studies indicate worse prognoses for mutated *KRAS* genes, while others do not[17,18]. This uncertain judgment suggests that the different codons of the *KRAS* gene, and even KRAS mutated variants, may have different associations with tumor development and responsiveness to systemic therapies. Lee *et al.* indicated that patients with mutated KRAS G13D or G12D had worse prognoses for 3-year DFS compared with KRAS wild-type patients in CC stage II-III (76% *vs.* 92%, p = 0.008 and 33% *vs.* 92%, p = 0.002, respectively). DFS was equal in mutated KRAS G12D and wild-type (86% *vs.* 92%, p = 0.61)[19].

Recently, the *KRAS* mutation rate in colorectal cancer has been reported in Viet Viet Nam but rarely in colon cancer. The rate of mut*ated* KRAS gene was 41.0% in colorectal cancer at any stage. More than 85% of mutations were diagnosed at Exon 2 (codon 12 or codo[3] 13)$. According to the study, *the* KRAS mutation rate was 44.9% (71/158) in CC stage II-**Table 1**II ($). The point mutation*s of* KRAS in Exons 2, 3, and 4 were determined, *and* KRAS mutations were mainly detected in Codon 12 or Codon 13. The KRAS variant of p.Gly12Asp (G12D) accounted for the highest ratio at 35.2%, followed by p.Gly13Asp (G13D; 21.1%); p.Gly12Val (G12V; 15.**Figure 1**%) ($). Several reports showed that the rate of mutated KRAS at G12D was higher than other point mutations. Hi*rose e*t al. reported that the more common KRAS mutations in 340 patients with metastatic colorectal cancers were KRAS G12D at 23.4% and KRAS G13D at 12.6%. Besides, KRAS G12V and G12C proportions were 21.2% and[20]4.7%$. According to Koulouridi et al., KRAS G12D, G12V, and G13D were more frequently detected (33.1%, 21.2%, and 16.7%, respecti[21]ely)$.

Different *KRAS* gene mutations have diverse effects on the biochemical and structural properties of the KRAS protein. Hence, specific KRAS mutations influence treatment outcomes differently in CC[7,22]. Results from a study in 200 CRCs with stage I-III indicated that the G12V and G12C mutations are related to worse DFS[23]. A larger retrospective analysis of five studies showed that KRAS G12C had the worst prognosis for overall survival (OS) and KRAS G13D had worse progression-free survival (PFS)[24]. However, in this study, no differences in survival were found in CC stage II-III according to the types of KRAS mutation. Although 3-year DFS and 4-year OS in KRAS p.Gly12Val (G12V) tend to be higher than KRAS wild-type (63.6% *vs.* 60.3%, p=0.881; 72.7% *vs.* 69.9%, p=0.962), the impact of the Gly to Val transitions at codon 12 on survival was not confirmed. The associations of the Gly to Asp transitions at *KRAS* gene codon 12 and 13 with survival were analyzed. The results indicated that variants of the KRAS gene were not a prognostic factor in CC stage II-III (**Table 2**).

The limitation of the research was the impact on survival, analyzed only by KRAS variants and not in association with other gene mutations such as BRAF, NRAS, and MMR.

CONCLUSIONS

This retrospective study indicated the rate of mutated KRAS variants and the insignificant impact of codon-specific KRAS mutations on survival outcomes in Vietnamese patients at stages II-III. KRAS p.Gly12Asp (G12D) and p.Gly13Asp (G13D) were more frequently confirmed in mutated KRAS patients, and this study has supplemented the clinical evidence to support the claim that codon-specific KRAS mutation is not a prognostic factor for survival in colon cancer stages II-III. The results contribute to a broader understanding of the prognostic value of KRAS mutations in early-stage colon cancer.

ABBREVIATIONS

AJCC - American Joint Committee on Cancer, **BRAF** - B-Raf Proto-Oncogene (Serine/Threonine Kinase), **CRC** - Colorectal Cancer, **DFS** - Disease-Free Survival, **EGFR** - Epidermal Growth Factor Receptor, **FFPE** - Formalin-Fixed, Paraffin-Embedded, **KRAS** - Kirsten Rat Sarcoma, **MAPK** - Mitogen-Activated Protein Kinase, **MMR** - Mismatch Repair, **NRAS** - Neuroblastoma RAS Viral Oncogene, **OS** - Overall Survival, **PFS** - Progression-Free Survival, **SPSS** - Statistical Package for the Social Sciences

ACKNOWLEDGMENTS

None.

AUTHOR'S CONTRIBUTIONS

Hoang Minh Cuong, Vu Hong Thang was responsible for the conceptualization, data acquisition, formal analysis, and writing of the original draft. Hoang Minh Cuong, Vu Hong Thang, Tran Bao Ngoc,

Nguyen Thuan Loi were responsible for data collection and investigation. Nguyen Thuan Loi was responsible for the critical review of the manuscript. All authors read and approved the final manuscript.

FUNDING

None.

AVAILABILITY OF DATA AND MATERIALS

Data and materials used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was permitted by the Ethics Committee of Hanoi Medical University (The approval number: NCS28/HMU-IRB).

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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