Observational Study

Medicine

OPEN

Epidemiological and clinicopathological features of *KRAS*, *NRAS*, *BRAF* mutations and MSI in Chinese patients with stage I–III colorectal cancer

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Abstract

The selection of appropriate treatment modalities based on the presence or absence of mutations in *KRAS*, *NRAS*, *BRAF*, and the microsatellite instability (MSI) status has become a crucial consensus in colorectal cancer (CRC) therapy. However, the distribution pattern of these genetic mutations and the prevalence of MSI status in Chinese stage I–III CRCs remain unclear. We retrospectively analyzed clinicopathological features, mutations in the *KRAS*, *NRAS*, and *BRAF* genes, as well as MSI status of 411 patients with stage I–III CRC who underwent surgery from June 2020 to December 2022 in the First Affiliated Hospital of Nanjing Medical University. The mutation rates of *KRAS*, *NRAS*, and *BRAF* were 48.9%, 2.2%, and 3.2%, respectively, and the microsatellite instability-high rate was 9.5%. KRAS mutation was independently associated with mucinous adenocarcinoma. Multivariate analysis suggested that tumor location and mucinous adenocarcinoma were independently associated with *BRAF* mutation. Only T stage was associated with *NRAS* mutations in the univariate analysis. Multivariate analysis revealed that factors such as larger tumor size, tumor location, younger age, and poor differentiation were independently associated with microsatellite instability-high status. The results illustrate the mutation frequencies of *KRAS*, *NRAS*, *BRAF* genes and MSI status in stage I–III CRC from the eastern region of China. These findings further validate the associations between these genes status and various clinicopathological characteristics.

Abbreviations: CRC = colorectal cancer, MSI = microsatellite instability, MSI-H = microsatellite instability-high, MSS = microsatellite stable, PCR = polymerase chain reaction.

Keywords: Chinese population, clinicopathological feature, colorectal cancer, genetic mutation, MSI

1. Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide, following lung and breast cancer.[1] According to the latest phase of national cancer statistics released by the National Cancer Center, the number of new cancer cases in China in 2016 was 4,064,000, of which 408,000 were CRC.[2] Precise selection of therapeutic approaches based on the primary tumor site and molecular pathology classification has emerged as a critical consensus in CRC management. Key biomarkers such as *KRAS*, *NRAS*, *BRAF* mutations, and microsatellite instability (MSI) status play a pivotal role in guiding treatment decisions. While the frequency of these genes has been extensively studied worldwide, their prevalence may exhibit variations across diverse regions and ethnicities among CRC patients.[3] However, there is currently a lack of research on these genes mutation profiles and distributions among Chinese patients with stage I-III CRC. Moreover, the relationship between *RAS* genes and MSI status in this population still lacks consistent conclusions and requires further investigation.

We performed a retrospective study of CRC cases with *KRAS*, *NRAS*, *BRAF*, and MSI data from the First Affiliated Hospital of Nanjing Medical University over the past 2 years to investigate the relationship between clinicopathological features and these specific genes, and our findings may provide guidance for the development of clinical strategies for genetic testing and personalized therapy in CRC patients.

2. Materials and methods

2.1. Design and data collection

A total of 411 patients who underwent radical resection of stage I–III CRC in The First Affiliated Hospital of Nanjing Medical University between June 2020 and December 2022

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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How to cite this article: Wang W, Wang R, Han X, Zhang W, Zhu L, Gu Y. Epidemiological and clinicopathological features of KRAS, NRAS, BRAF mutations and MSI in Chinese patients with stage I–III colorectal cancer. Medicine 2024;103:14(e37693).

Received: 6 November 2023 / Received in final form: 23 February 2024 / Accepted: 1 March 2024

http://dx.doi.org/10.1097/MD.0000000000037693

were included in this study. Patients were selected based on specific inclusion criteria, which included: 1. Patient first diagnosed with colorectal cancer and underwent a radical resection. 2. Stage I–III colorectal adenocarcinoma diagnosed by postoperative pathology. 3. With complete postoperative pathology information. 4. Postoperative tissue samples tested for *KRAS*, *NRAS*, *BRAF* mutations and MSI status. We reviewed pathological records and the medical record system to gather the following information: patient gender, age, tumor location, whether preoperative chemoradiotherapy was given, and morphological characteristics (histological type, tumor differentiation, tumor penetration depth, lymph node involvement, extranodal tumor deposits, lymphatic or vascular invasion, and perineural invasion). Our study was conducted in accordance with the principles outlined in the Declaration of Helsinki and was approved by the Ethics Commission of The First Affiliated Hospital of Nanjing Medical University (approval number: 2020-SRFA-080). All patients have provided written consent for the utilization of their tumor tissue samples for molecular analysis.

2.2. KRAS/NRAS/BRAF mutations detection

Mutation detection was carried out on formaldehyde-fixed, paraffin-embedded tissues following confirmation by 2 pathologists through examination of hematoxylin and eosin-stained slides. Tumoral DNA was extracted from selected formalin-fixed, paraffin-embedded specimens using the QIAamp DNA formalin-fixed, paraffin-embedded Tissue Kit (Qiagen, Germany). *KRAS*, *NRAS*, and *BRAF* molecular testing was performed using the ADx *KRAS/NRAS/BRAF* Mutation Analysis Panel Kit (AmoyDx, Xiamen, China). The mutational status of *KRAS* (exon 2: codons 12 and 13, exon 3: codon 61, and exon 4: codons 117 and 146), *NRAS* (exon 2: codon 12, and exon 3: codon 61), and *BRAF* (exon 15: codon 600) was analyzed. The total reaction volume is 35 μL, including 5 μL of template DNA and 30 μL of reaction mixture (*KRAS*, *NRAS* or *BRAF* polymerase chain reaction [PCR] reaction solution, *KRAS*, *NRAS* or *BRAF* primer-probe mix, Taq enzyme solution) PCR amplification were performed using ABI 7500 as follows: denaturation for 5 minutes at 95 °C; 25 seconds at 95 °C, 20 seconds at 64 °C, 20 seconds at 72 °C for 15 cycles; 25 seconds at 93 °C, 35 seconds at 60 °C, 20 seconds at 72 °C for 31 cycles; FAM and VIC signals were collected at 60 °C for 31 cycles. We defined a Ct value of <29 as mutation positive, whereas a Ct value ≥29 as negative.

2.3. MSI detection

MSI was assessed using the National Cancer Institute 2B3D panel, which includes BAT-25, BAT-26, D2S123, D5S346, and D17S250 markers. The MSI Test Kit from YuanQi in Shanghai, China was used for the detection, employing polymerase chain reaction and capillary electrophoresis. The assay was conducted on a BIO-RAD 1000 PCR system (BIO-RAD, CA) following the manufacturer's instructions. The parameters used were as follows: denaturation for 5 minutes at 95 °C; 30 seconds at 95 °C, 90 seconds at 60 °C, 60 seconds at 72 °C for 30 cycles; 10 minutes at 72 °C and thereafter held at 4 °C. After denaturing at 95 °C and 4 °C for 5 minutes. After amplification, samples were analyzed by capillary electrophoresis using an ABI-3500Px (ABI). Microsatellite instability-high (MSI-H) was defined as the presence of instability in at least 2 microsatellite markers, while MSI-low was defined as the presence of instability in 1 microsatellite marker. Microsatellite stable (MSS) was defined as the absence of any instability.

2.4. Statistical analysis

The percentages were used to summarize certain clinical and pathological characteristics. Statistical analyses were conducted using SPSS version 26.0 (IBM Corporation, Armonk, NY). Chi-squared tests or Fisher exact tests for categorical variables were used to assess the correlation between the gene mutation status and clinical features. For continuous variables, the Kolmogorov–Smirnov test was performed to verify the normal distribution assumptions. The exploratory comparison of normally distributed and nonnormally distributed independent groups was performed using *t*-tests and Mann–Whitney U tests (2 groups). $P < .05$ was considered statistically significant.

3. Results

3.1. Patient characteristics

Table 1 summarizes basic clinical characteristics of 411 patients enrolled in the study. The study cohort consisted of 255 men (62.0%) and 156 women (38.0%), with an average age of 62 years. The majority of patients were from eastern regions of China. Among the participants, 238 out of the 411 patients (57.9%) were diagnosed with primary tumors in the colon. Specifically, 23.1% of these tumors were located in the ascending colon, while 34.8% were situated in the descending colon. Additionally, a total of 173 patients (42.1%) had primary tumors located in the rectum. 51 (12.4%) of the patients had mucinous adenocarcinoma, whereas the rest had non-mucinous adenocarcinomas. Among the 411 patients, 334 (81.3%) tumors were classified as grade I–II, 77 (18.7%) as grade III–IV. The majority of patients were diagnosed with T3 stage (74.2%), while

Table 1

Clinical characteristics of 411 patients.

Variables		N (%)
Sex	Male	255 (62.0%)
	Female	156 (38.0%)
Age		61.72 ± 12.72
Tumor site	Ascending colon	95 (23.1%)
	Descending colon	143 (34.8%)
	Rectum	173 (42.1%)
Tumor size (cm)		4.27 ± 1.73
Neoadjuvant therapy	No	372 (90.5%)
	Yes	39 (9.5%)
Mucinous carcinoma	No	360 (87.6%)
	Yes	51 (12.4%)
Differentiation	G1–G2	334 (81.3%)
	G3–G4	77 (18.7%)
T stage	1	15 (3.6%)
	2	49 (11.9%)
	3	305 (74.2%)
	4	42 (10.2%)
Lymph node metastasis	Negative	215 (52.3%)
	Positive	196 (47.7%)
TNM stage	1	53 (12.9%)
	2	162 (39.4%)
	3	196 (47.7%)
Extranodal tumor deposit	Negative	339 (82.5%)
	Positive	72 (17.5%)
Perineural invasion	Negative	301 (73.2%)
	Positive	110 (26.8%)
Lymphovascular invasion	Negative	185 (45.0%)
	Positive	226 (55.0%)
KRAS mutant		201 (48.9%)
NRAS mutant		9 (2.2%)
BRAF mutant		13 (3.2%)
MSI-H		39 (9.5%)

patients with stage T1, T2, and T4 accounted for 3.6%, 11.9%, and 10.2%, respectively.196 patients (47.7%) displayed locoregional lymph node metastases. All patients included in the study were non-stage IV cancer patients. Among them, the majority had stage III disease, accounting for 47.7%. The percentages of stage I and II patients were 12.9% and 39.4%, respectively. Most patients (82.5%) showed no extranodal tumor deposit. Additionally, 301 (73.2%) patients had no perineural invasion, whereas 226 (55.0%) patients presented with lymphovascular invasion. *KRAS*, *NRAS*, and *BRAF* mutations were identified in 48.9%, 2.2%, and 3.2% of these 411 patients, respectively. Moreover, 39 (9.5%) of the patients exhibited MSI-H.

3.2. KRAS gene mutations and correlations with clinicopathological features

Univariate analyses of clinicopathologic features according to *KRAS* status are listed in Table 2. In univariate analysis, factors contributing to the high mutation rate of *KRAS* include larger tumor size, tumor site and mucinous adenocarcinoma. In addition, potentially meaningful clinical variables were also included in the following multivariate analysis.[4,5] Multivariate analysis of clinicopathologic features according to mutations in *KRAS* is shown in Table 3. The results indicate that a high *KRAS* mutation rate was independently associated with mucinous adenocarcinoma.

3.3. Spectrum of KRAS mutations

We further analyzed the spectrum of *KRAS* alterations. Since a combined human *KRAS/NRAS/BRAF* mutation detection kit was used in our study, all other *KRAS* locus mutations except G13D were detected in a mixed mode. The distribution of *KRAS* mutations in the 201 patient samples is shown in Figure 1. The results showed that among *KRAS* mutations, G12D/G12S accounted for 34.3%, G13D for 17.4%, G12C/G12V/G12A/G12R/G13C for 26.4%, Q61L/Q61R/Q61H for 6.5%, A59T/Q61K for 1.5%, and K117N/A146VI/A146P/A146T for 14.4% of all *KRAS* mutations.

3.4. BRAF mutations and correlations with clinicopathological features

The results from univariate highlight a potential relationship between the *BRAF* mutation rate and tumor location, as well as mucinous adenocarcinoma. The multivariate analysis further strengthens these associations by demonstrating that these factors are independently associated with *BRAF* mutations. Specifically, ascending colon tumors were found to have higher rates of *BRAF* mutations. Additionally, mucinous carcinoma showed a higher incidence of *BRAF* mutations compared to other histological types. Results of analysis are summarized in Tables 2 and 3.

3.5. NRAS gene mutations and correlations with clinicopathological features

In the univariate analysis, only T stage showed a significant association with *NRAS* mutations. As a result, *NRAS* mutations were excluded from the multivariate analysis (Table 2).

3.6. MSI status and correlations with clinicopathological features

The univariate analysis revealed that a high rate of MSI-H was associated with several factors, including younger age, larger tumor size, tumor location, mucinous adenocarcinoma, poor differentiation, advanced TNM stage, absence of lymph node metastasis, and no lymphovascular invasion.

Subsequently, the multivariate analysis was performed to assess the independent contributions of these factors to the occurrence of MSI-H (as shown in Tables 4 and 5). The results of the multivariate analysis indicated that larger tumor size, tumor location, younger age, and poor differentiation were independently and significantly correlated with a higher incidence of MSI-H.

4. Discussion

The incidence of CRC has been increasing worldwide, making it crucial to gain a better understanding of the factors contributing to CRC tumorigenesis. A rich history of investigations have validated that the progression and prognosis of CRC involves key genetic mutations, including *KRAS*, *NRAS*, *BRAF*, as well as MSI status.[6,7]

KRAS, as the most common typical functional mutation in CRC, has been extensively studied in recent years. It belongs to the family of GTP/GDP binding proteins with GTPase activity, which plays a role in transmitting mitogenic signals from the cell membrane to the nucleus. Mutations in *KRAS* attenuate the intrinsic GTPase activity of RAS protein, resulting in prolonged activation of RAS. About 40% of CRC patients carry activating missense mutations in *KRAS* and most of them occurring at codons 12, 13, and 61.[8] Numerous studies have indicated the association between *KRAS* mutations and the occurrence, progression and recurrence of CRC. Moreover, it has been well-established that *KRAS* mutations are associated with resistance to anti-epidermal growth factor receptor therapies, such as cetuximab and panitumumab.[9] Therefore, the identification of *KRAS* mutations in CRC patients is crucial for guiding treatment decisions and avoiding ineffective therapies.

Although it was widely believed that the mutation rate of *KRAS* in CRC was around 40%, there remains a lack of conclusions regarding whether the *KRAS* mutation rate is consistent across different regions in CRC populations. Some studies have been conducted to investigate the prevalence of *KRAS* mutations in CRC across different regions of China. Table 6 presents studies on *KRAS* mutation rates in colorectal cancer populations in different regions of China, reporting rates ranging from 36.5% to 52.7%.[3,5,10–12] The variation in rates may be attributed to differences in target populations and the range of *KRAS* mutation sites covered by the detection methods used in the studies. We observed a significant increase in the *KRAS* mutation rate in colorectal cancer as more mutation sites of *KRAS* were included in the detection. In our study, we identified *KRAS* mutations in 201 (48.9%) out of 411 stage I–III CRC patients from the eastern regions of China, which is higher compared to some of the aforementioned studies. In contrast to these studies, our study includes not only the common mutation sites on exons 2/3 of *KRAS* but also the mutation sites on exon 4, which may allow us to identify more patients with *KRAS* mutations (Table 7). Our result was consistent with the studies of Guo TA and Fang Guo. Both studies reported *KRAS* mutation rates exceeding 45%, and both analyzed mutation sites on *KRAS* exons 2, 3, and 4.[5,12] Accordingly, for *KRAS* mutation testing, it is necessary to cover all possible clinically relevant mutation sites. It can help identify the patients with rare mutations, allowing for more accurate guidance for treatment decisions.

In our study, univariate analysis showed that *KRAS* mutation was significantly associated with tumor size, site and mucinous adenocarcinoma, which is consistent with previous reports. We observed a notable decrease in the *KRAS* mutation rate from the right colon to the left colon, with a subsequent increase in the rectum. Specifically, the mutation rate of *KRAS* in the right colon was found to be 56.8%, in the left colon was 37.8%, and in the rectum was 53.8%. These findings are consistent

Table 2

Univariate analysis of genetic mutations and clinicopathological characteristics.

Variables	*KRAS* Wild-type N = 210	*KRAS* Mutant N = 201	*P*-value	*NRAS* Wild-type N = 402	*NRAS* Mutant N = 9	*P*-value	*BRAF* Wild-type N = 398	*BRAF* Mutant N = 13	*P*-value
Sex			.612			.452			1.000
Male	133 (52.2%)	122 (47.8%)		251 (98.4%)	4 (1.6%)		247 (96.9%)	8 (3.1%)	
Female	77 (49.4%)	79 (50.6%)		151 (96.8%)	5 (3.2%)		151 (96.8%)	5 (3.2%)	
Age	60.82 ± 13.01	62.66 ± 12.37	.143	61.64 ± 12.82	65.44 ± 6.17	.375	61.83 ± 12.77	58.46 ± 10.99	.348
Tumor size (cm)	4.09 ± 1.72	4.46 ± 1.71	.027	4.28 ± 1.73	4.03 ± 1.67	.677	4.29 ± 1.74	3.58 ± 1.20	.141
Tumor site			.004			.569			.002
Ascending colon	41 (43.2%)	54 (56.8%)		92 (96.8%)	3 (3.2%)		87 (91.6%)	8 (8.4%)	
Descending colon	89 (62.2%)	54 (37.8%)		141 (98.6%)	2 (1.4%)		139 (97.2%)	4 (2.8%)	
Rectum	80 (46.2%)	93 (53.8%)		169 (97.7%)	4 (2.3%)		172 (99.4%)	1 (0.6%)	
Neoadjuvant therapy			.867			1.000			.223
No	191 (51.3%)	181 (48.7%)		363 (97.6%)	9 (2.4%)		362 (97.3%)	10 (2.7%)	
Yes	19 (48.7%)	20 (51.3%)		39 (100.0%)	0 (0.0%)		36 (92.3%)	3 (7.7%)	
Mucinous carcinoma			.007			.528			.014
No	193 (53.6%)	167 (46.4%)		351 (97.5%)	9 (2.5%)		352 (97.8%)	8 (2.2%)	
Yes	17 (33.3%)	34 (66.7%)		51 (100.0%)	0 (0.0%)		46 (90.2%)	5 (9.8%)	
Differentiation			.058			.117			.442
G1–G2	163 (48.8%)	171 (51.2%)		329 (98.5%)	5 (1.5%)		325 (97.3%)	9 (2.7%)	
G3–G4	47 (61.0%)	30 (39.0%)		73 (94.8%)	4 (5.2%)		73 (94.8%)	4 (5.2%)	
T stage			.225			.010			.381
1	10 (66.7%)	5 (33.3%)		15 (100.0%)	0 (0.0%)		14 (93.3%)	1 (6.7%)	
2	22 (44.9%)	27 (55.1%)		47 (95.9%)	2 (4.1%)		49 (100.0%)	0 (0.0%)	
3	152 (49.8%)	153 (50.2%)		302 (99.0%)	3 (1.0%)		294 (96.4%)	11 (3.6%)	
4	26 (61.9%)	16 (38.1%)		38 (90.5%)	4 (9.5%)		41 (97.6%)	1 (2.4%)	
TNM stage			.133			.349			.278
1	23 (43.4%)	30 (56.6%)		51 (96.2%)	2 (3.8%)		52 (98.1%)	1 (1.9%)	
2	77 (47.5%)	85 (52.5%)		160 (98.8%)	2 (1.2%)		159 (98.1%)	3 (1.9%)	
3	110 (56.1%)	86 (43.9%)		191 (97.4%)	5 (2.6%)		187 (95.4%)	9 (4.6%)	
Lymph node metastasis			.061			.888			.158
Negative	100 (46.5%)	115 (53.5%)		211 (98.1%)	4 (1.9%)		211 (98.1%)	4 (1.9%)	
Positive	110 (56.1%)	86 (43.9%)		191 (97.4%)	5 (2.6%)		187 (95.4%)	9 (4.6%)	
Extranodal tumor deposit			.605			.088			0.869
Negative	171 (50.4%)	168 (49.6%)		334 (98.5%)	5 (1.5%)		329 (97.1%)	10 (2.9%)	
Positive	39 (54.2%)	33 (45.8%)		68 (94.4%)	4 (5.6%)		69 (95.80%)	3 (4.2%)	
Perineural invasion			.657			.945			1.000
Negative	156 (51.8%)	145 (48.2%)		295 (98.0%)	6 (2.0%)		291 ((96.7%)	10 (3.3%)	
Positive	54 (49.1%)	56 (50.9%)		107 (97.3%)	3 (2.7%)		107 (97.3%)	3 (2.7%)	
Lymphovascular invasion			.061			.709			.399
Negative	85 (45.9%)	100 (54.1%)		182 (98.4%)	3 (1.6%)		181 (97.8%)	4 (2.2%)	
Positive	125 (55.3%)	101 (44.7%)		220 (97.3%)	6 (2.7%)		217 (96.0%)	9 (4.0%)	
MSI			.046			1.000			1.000
MSS/MSI-L	196 (52.7%)	176 (47.3%)		363 (97.6%)	9 (2.4%)		360 (96.8%)	12 (3.2%)	
MSI-H	14 (35.9%)	25 (64.1%)		39 (100.0%)	0 (0.0%)		38 (97.4%)	1 (2.6%)	

with previous research, which also reported similar trends in *KRAS* mutation distribution along the colorectal tract.[5] Many published research data showed that the *KRAS* and *BRAF* mutation occurred more frequently in mucinous CRC than in non-mucinous CRC.[13,14] In this study, we found that 66.7% of patients with mucinous adenocarcinoma had *KRAS* mutations, which is significantly higher than that in non-mucinous pathological types (46.4%). A few possible explanations for this phenomenon exist. Firstly, mucinous CRC tends to be predominantly located in the proximal colon, which has been demonstrated to have a higher *KRAS* mutation rate. In addition, from another perspective, *KRAS* mutations are more likely to stimulate the production of mucin in tumor tissues. In the multivariate analysis, when considering all the factors together, only mucinous adenocarcinoma remained as an independent correlative factor, indicating that it has a stronger and more direct relationship with the presence of *KRAS* mutation.

As we all know, *KRAS* exon 2 is associated with poor prognosis and resistance to epidermal growth factor receptor therapy,[15–17] whereas *KRAS* exon 3 and exon 4 mutations have not been widely tested due to low mutation rates. Thus, the prognostic value of mutations on *KRAS* exon 3/4 remains unclear. Our study further retrospectively analyzed relationships between *KRAS* exon 2/3/4 mutations and clinicopathological features (Table 7). Unfortunately, analysis showed few significant associations in different exons. Perhaps due to the limited number of patients, a larger clinical study is needed in the future to explore whether there is a possible association with clinical features and prognosis. We further analyzed the spectrum of *KRAS* alterations. We observed that the G12D/G12S mutations had the highest frequency, approximately 34.3%. This finding indicates that the G12D mutation may predominantly contribute to this high mutation rate, as the reported mutation rate of G12S is only about 4.8%.[18] Additionally, the G13D mutation accounted for 17.4% of all *KRAS* mutations, which aligns with a previous study conducted in China.[19] In the future, as more KRAS inhibitors that target specific point mutations are discovered, it will become increasingly crucial to accurately identify the specific mutation sites of KRAS in patients.

Compared to *KRAS*, *NRAS*, exhibits a lower mutation rate in CRC. Previous studies have shown that the frequency of *NRAS*

Table 3

Multivariate analysis of genetic mutations and clinicopathological characteristics.

	KRAS mutation			*BRAF* mutation		
Variables	***P*-value**	**OR**	**95% CI**	***P*-value**	**OR**	**95%CI**
Sex (female)	.530	1.142	0.754–1.731	.642	0.753	0.228–2.486
Age	.075	1.015	0.999–1.032			
Tumor size (cm)	.202	1.091	0.955–1.246			
Mucinous carcinoma	.024	2.111	1.105–4.034	.040	3.573	1.060–12.042
Extranodal tumor deposit	.955	0.985	0.576–1.683	.857	1.150	0.253–5.214
Lymphovascular invasion				.489	1.602	0.421–6.092
MSI-H	.152	1.778	0.810–3.905			
Differentiation						
G1–G2					1	Ref
G3–G4				.740	1.245	0.342–4.535
Tumor site						
Ascending colon		1	Ref		1	Ref
Descending colon	.052	0.577	0.331–1.005	.135	0.378	0.106–1.355
Rectum	.441	1.247	0.712–2.183	.020	0.080	0.009–0.667

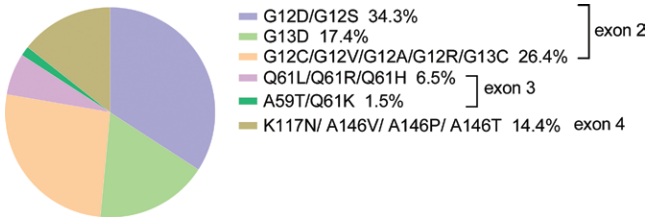

Figure 1. Frequency of each codon in *KRAS* mutations. Among *KRAS* mutations, G12D/G12S accounted for 34.3%, G13D for 17.4%, G12C/G12V/G12A/G12R/G13C for 26.4%, Q61L/Q61R/Q61H for 6.5%, A59T/Q61K for 1.5%, and K117N/A146VI/A146P/A146T for 14.4%.

mutations in CRC samples ranges from 2.2% to 4%.[20] Guo TA reported that the mutation rate of *NRAS* is 3.2%. In our study, we identified 9 cases of *NRAS* mutations out of 411 patients, which reflects a mutation rate of 2.2%. The results of the 2 studies are very similar. The rare mutation rate has hindered the identification of pathological and clinical correlation of *NRAS* mutated CRC.

Besides *KRAS* and *NRAS*, *BRAF* is another important mutation target in CRC. In metastatic CRC, the reported *BRAF* mutation rate ranges from 8% to 12% and is associated with poor prognosis.[21] In *BRAF*, the $BRAF^{V600E}$ mutation is the most commonly observed alteration, accounting for up to 90% of all *BRAF* mutations.[22] Our study dedicated that $BRAF^{V600E}$ mutation rate was 3.2%, which was consistent with literature reports in Chinese patients.[5,12] A meta-analysis conducted by Dong Chen revealed that $BRAF^{V600E}$ mutation in CRC was significantly associated with various clinicopathological factors, including female gender, advanced age, poor differentiation, mucinous histology, proximal colon tumor location and MSI.[23] In our study, multivariate analysis revealed that mucinous carcinoma (OR = 3.573, 95%CI: 1.060–12.042, P = .04) were independently associated with the $BRAF^{V600E}$ mutation. This finding confirmed that the $BRAF^{V600E}$ mutation in CRC was significantly associated with mucinous histology. Of 51 patients with mucinous histology, 5 (9.8%) were identified to harbor $BRAF^{V600E}$ mutation, whereas 8 (2.2%) of 360 patients with non-mucinous histology were $BRAF^{V600E}$ mutation positive. Our data showed that the rate of $BRAF^{V600E}$ mutations in the ascending colon was 8.4%, while in the left colon and rectum, the rates were 2.8% and 0.6%, respectively. The multivariate analysis also suggested that $BRAF^{V600E}$ mutations were less likely to occur in the rectum (OR = 0.08, 95%CI: 0.009–0.667, P = .02).

In CRC, MSI is recognized as one of the main carcinogenic pathways and is a predictive marker for the efficacy of immune checkpoint inhibitors.[24] It is also a molecular marker of Lynch syndrome, which is a type of hereditary nonpolyposis CRC. MSI is also used for prognosis prediction and treatment guidance in CRC.[25] In stage II CRC, patients with MSI-H have a better prognosis but do not benefit from adjuvant 5-FU therapy. Multiple previous studies have consistently demonstrated that MSI is more common in the right colon.[26] Our study confirmed this phenomenon. We found that the proportion of right colon cancer in MSI-H group was as high as 23/39 (59.0%), much higher than in the MSS group 72/372 (19.4%). Furthermore, we observed that the proportion of patients with rectal cancer in the MSS/MSI-L group was 169/372 (45.4%), which was higher than the 4/39 (10.2%) observed in the MSI-H group. These findings suggest that MSI may be involved in the development of right colon cancer, and the mechanisms underlying the development of left and right colon cancers may differ at the genetic level. This study showed that the MSI-H group had earlier TNM stage compared to the MSS/MSI-L group, with 31/39 (79.5%) *vs* 184/372 (49.5%) of patients categorized as stage I–II, and a lower rate of lymph node metastasis. This may partially explain why MSI-H is associated with a better prognosis. Previous research indicated that MSI-H is more common in elderly females attributed to higher prevalence of CpG island methylator phenotype and *MLH1* hypermethylation.[27] However, the findings of our study differ from this. In fact, our data demonstrate that the occurrence of MSI is higher in younger patients. This result aligns with a previous study conducted by Serebriiskii IG,[28] highlighting the existence of differences between various research investigations. A great production of mucin with extracellular accumulation often correlates with MSI,[26] consistent with this finding, our results indicate that CRC with MSI-H are more likely to exhibit poor differentiation, have a larger diameter, and be characterized by mucinous adenocarcinoma, compared to cancers with MSS/MSI-L. These findings are consistent with the studies conducted by Gryfe R and Kaur G.[29,30]

Rajagopalan H's and Blaker H's studies all demonstrated that MSI-H CRCs have a significantly higher incidence of *BRAF* mutations and a lower incidence of *KRAS* mutations compared to MSS CRCs.[31,32] However, in our study, we did not observe any correlation between MSI status and $BRAF^{V600E}$ mutations. The frequency of $BRAF^{V600E}$ mutation observed in patients with MSI-H was 1/39 (2.6%), while in MSS tumors it was 12/372 (3.2%). Another recent study conducted among the Chinese population also failed to confirm this phenomenon.[33] Inconsistent results of these studies may be due to differences in the stage of the population included, sample size, and the proportion of patients with Lynch syndrome. Since the occurrence rates of both $BRAF^{V600E}$ mutation and MSI-H are relatively low,

Table 4

Univariate analysis of MSI states and clinicopathological characteristics.

		MSI		
		MSS/MSS-L	MSI-H	
Variables		**N = 372**	**N = 39**	***P*-value**
Sex	Male	233 (91.4%)	22 (8.6%)	.489
	Female	139 (89.1%)	17 (10.9%)	
Age		62.34 ± 12.30	55.79 ± 15.14	.002
Tumor size (cm)		4.07 ± 1.53	6.154 ± 2.32	<.001
Tumor site	Ascending colon	72 (75.8%)	23 (24.2%)	<.001
	Descending colon	131 (91.6%)	12 (8.4%)	
	Rectum	169 (97.7%)	4 (2.3%)	
Neoadjuvant therapy	No	334 (89.8%)	38 (10.2%)	.206
	Yes	38 (97.4%)	1 (2.6%)	
Mucinous carcinoma	No	332 (92.2%)	28 (7.8%)	.004
	Yes	40 (78.4%)	11 (21.6%)	
Differentiation	G1–G2	309 (92.5%)	25 (7.5%)	.006
	G3–G4	63 (81.8%)	14 (18.2%)	
T stage	1	15 (100.0%)	0 (0.0%)	.142
	2	46 (93.9%)	3 (6.1%)	
	3	270 (88.5%)	35 (11.5%)	
	4	41 (97.6%)	1 (2.4%)	
TNM stage	1	50 (94.3%)	3 (5.7%)	<.001
	2	134 (82.7%)	28 (17.3%)	
	3	188 (95.9%)	8 (4.1%)	
Lymph node metastasis	Negative	184 (85.6%)	31 (14.4%)	<.001
	Positive	188 (95.9%)	8 (4.1%)	
Extranodal tumor deposit	Negative	303 (89.4%)	36 (10.6%)	.119
	Positive	69 (95.8%)	3 (4.2%)	
Perineural invasion	Negative	269 (89.4%)	32 (10.6%)	.254
	Positive	103 (93.6%)	7 (6.4%)	
Lymphovascular invasion	Negative	158 (85.4%)	27 (14.6%)	.002
	Positive	214 (94.7%)	12 (5.3%)	
KRAS mutant	Negative	196 (93.3%)	14 (6.7%)	.046
	Positive	176 (87.6%)	25 (12.4%)	
BRAF mutant	Negative	360 (90.5%)	38 (9.5%)	1.000
	Positive	12 (92.3%)	1 (7.7%)	
NRAS mutant	Negative	363 (90.3%)	39 (9.7%)	1.000
	Positive	9 (100%)	0 (0.0%)	

Table 5

Multivariate analysis of MSI state and clinicopathological characteristics.

Variables	***P*-value**	**OR**	**95%CI**
Age	.003	0.953	0.923–0.984
Tumor size (cm)	<.001	1.563	1.254–1.949
Mucinous carcinoma	.334	1.650	0.597–4.566
Lymphovascular invasion	.532	0.626	0.144–2.723
Lymph node metastasis	.331	0.343	0.040–2.962
TNM Stage	.625	0.687	0.152–3.100
KRAS mutant (positive)	.161	1.869	0.780–4.481
Differentiation			
G1–G2		1	Ref
G3–G4	.021	3.285	1.200–8.987
Tumor site			
Ascending colon		1	Ref
Descending colon	.022	0.340	0.135–0.859
Rectum	<.001	0.100	0.027–0.364

the correlation between MSI status and $BRAF^{V600E}$ mutations may only become apparent with a sufficiently large sample size. Furthermore, our result showed that the mutation rate of *KRAS* in the MSI-H group was 25/39 (64.1%), which was significantly higher than the rate of 176/372 (47.3%) in the MSI-L/MSS group. Previous study have suggested that MSI tumors have fewer *KRAS* mutations than MSS tumors.[34] However, our finding is inconsistent with this report. Recently, a study reported that Lynch dMMR/MSI-H tumors more often harbored a *RAS* mutation (65%),[35] suggesting that the results observed in our study may be due to a potentially higher proportion of Lynch syndrome patients in the MSI-H group. The MMR gene status detection is crucial for the diagnosis of Lynch syndrome. However, the majority of the patients in our study had not undergone MMR gene status testing. Therefore, we are not able to accurately identify the patients with Lynch syndrome. Interestingly, we found that in the MSI-H group, mutations on exon 4 (including K117N/A146V/A146P/A146T) accounted for up to 28% of all *KRAS* mutations, which is higher than the 12.5% observed in the MSI-L/MSS group. The mutation rate of 28% closely matches the results of the aforementioned study, where the A146 mutation rate reached 20% in the MSI-H group.[35] The high frequency of K117N/A146V/A146P/A146T mutations in the MSI-H group of our study may be one of the critical factors contributing to the overall increased *KRAS* mutation rate. Overall, it suggests that the exact relationship between *KRAS* mutations and MSI in the real world still requires further research to be determined.

In summary, our study suggests MSI-H CRC exhibit distinct clinical and pathological characteristics, including a proximal location, poor differentiation, larger tumor size, and younger patients. There were some limitations in our study. Firstly, the sample size was relatively small. For rare gene mutations like *NRAS*, there were very few corresponding mutant patents included in the study, making it difficult to conduct further

Table 6

***KRAS* mutation frequencies in reported studies.**

First author	Location of study	Study period	Total patients	Study subjects	Codons detection sites	*KRAS* mutation frequency (%)
Ye JX et al[3]	Northern China	2010–2013	535	Stage I–IV	Exon 2	37.9%
Xu XM et al[9]	Northern China	2009–2010	52	Unknown	Exons 2/3	36.5%
Yang Q et al[10]	Northern China	2016–2018	226	Stage I–IV	Exon 2	39.8%
Guo TA et al[5]	Eastern China	2013–2018	1834	Stage I–IV	Exons 2/3/4	45.7%
Guo F et al[11]	Eastern China	2007–2012	353	Stage I–III	Exons 2/3/4	52.7%

Table 7

Univariate analysis of *KRAS* mutation sites and clinicopathological characteristics.

Variables		*KRAS* exon 2		*KRAS* exon 3		*KRAS* exon 4	
		N = 157	*P*-value	N = 16	*P*-value	N = 29	*P*-value
Sex			.209		.036		1
	Male	91 (35.7%)		14 (5.5%)		18 (7.1%)	
	Female	66 (42.3%)		2 (1.3%)		11 (7.1%)	
Age		63.23 ± 12.18	.059	67.81 ± 10.68	.051	57.07 ± 12.59	.041
Tumor size (cm)		4.34 ± 1.61	.534	4.64 ± 1.73	.386	5.13 ± 2.14	.030
Tumor site			.037		.359		.907
	Ascending colon	43 (45.3%)		4 (4.2%)		7 (7.4%)	
	Descending colon	43 (30.1%)		3 (2.1%)		9 (6.3%)	
	Rectum	71 (41.0%)		9 (5.2%)		13 (7.5%)	
Neoadjuvant therapy			1		.987		.623
	No	142 (38.2%)		15 (4.0%)		25 (6.7%)	
	Yes	15 (38.5%)		1 (2.6%)		4 (10.3%)	
Mucinous carcinoma			.169		.241		.090
	No	133 (36.9%)		12 (3.3%)		22 (6.1%)	
	Yes	24 (47.1%)		4 (7.8%)		7 (13.7%)	
Differentiation			.068		.328		.459
	G1-G2	135 (40.4%)		15 (4.5%)		22 (6.6%)	
	G3-G4	22 (28.6%)		1 (1.3%)		7 (9.1%)	
T stage			.304		.547		1.000
	1	3 (20.0%)		1 (6.7%)		1 (6.7%)	
	2	21 (42.9%)		3 (6.1%)		3 (6.1%)	
	3	120 (39.3%)		11 (3.6%)		22 (7.2%)	
	4	13 (31.0%)		1 (2.4%)		3 (7.1%)	
TNM stage			.366		.269		.377
	1	23 (43.4%)		4 ((7.5%)		3 (5.7%)	
	2	66 (40.7%)		4 (2.5%)		15 (9.3%)	
	3	68 (34.7%)		8 (4.1%)		11 (5.6%)	
Lymph node metastasis			.187		1		.336
	Negative	89 (41.4%)		8 (3.7%)		18 (8.4%)	
	Positive	68 (34.7%)		8 (4.1%)		11 (5.6%)	
Extranodal tumor deposit			.509		.839		.800
	Negative	132 (38.9%)		14 (4.1%)		23 (6.8%)	
	Positive	25 (34.7%)		2 (2.8%)		6 (8.3%)	
Perineural invasion			1		.900		.664
	Negative	115 (38.2%)		11 (3.7%)		20 (6.6%)	
	Positive	42 (38.2%)		5 (4.5%)		9 (8.2%)	
Lymphovascular invasion			.415		.799		.175
	Negative	75 (40.5%)		8 (4.3%)		17 (9.2%)	
	Positive	82 (36.3%)		8 (3.5%)		12 (5.3%)	
MSI			.703		1.000		.014
	MSS/MSI-L	141 (37.9%)		14 (3.8%)		22 (5.9%)	
	MSI-H	16 (41.0%)		2 (5.1%)		7 (17.9%)	

analysis. Therefore, future studies should strive to expand the sample size and include a larger population of individuals carrying rare gene mutations. Secondly, since the sampling period of this study is between June 2020 and December 2022, mature postoperative recurrence and overall survival data have not yet been generated. As a result, the prognosis could not be included in the analysis. In future studies, we will maintain follow-up with this patient cohort to conduct a more in-depth analysis of the impact of mutations in *KRAS*, *NRAS*, *BRAF*, and MSI status.

5. Conclusions

We systematically described and statistically analyzed the frequencies and distributions of *KRAS*, *NRAS*, *BRAF* genetic mutations, MSI status and their relationship in 411 cases of stage I–III CRC in eastern regions of China. We confirmed that the *KRAS*, *NRAS*, *BRAF* mutation and MSI-H are associated with several clinicopathological characteristics, which may provide more valuable insights for personalized genetic tests in the future.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (82072675).

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