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ORIGINAL ARTICLE

Basic Study

Comprehensive analysis of gene mutations and mismatch repair in Chinese colorectal cancer patients

Huang Chen, Rui-Ying Jiang, Zhan Hua, Xiao-Wei Wang, Xiao-Li Shi, Ye Wang, Qian-Qian Feng, Jie Luo, Wu Ning, Yan-Fen Shi, Da-Kui Zhang, Bei Wang, Jian-Zheng Jie, Ding-Rong Zhong

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Abstract

BACKGROUND

RAS, *BRAF*, and mismatch repair (MMR)/microsatellite instability (MSI) are crucial biomarkers recommended by clinical practice guidelines for colorectal cancer (CRC). However, their characteristics and influencing factors in Chinese patients have not been thoroughly described.

AIM

To analyze the clinicopathological features of *KRAS*, *NRAS*, *BRAF*, and *PIK3CA* mutations and the DNA MMR status in CRC.

METHODS

We enrolled 2271 Chinese CRC patients at the China-Japan Friendship Hospital. MMR proteins were tested using immunohistochemical analysis, and the *KRAS/NRAS/BRAF/PIK3CA* mutations were determined using quantitative polymerase chain reaction. Microsatellite status was determined using an MSI detection kit. Statistical analyses were conducted using SPSS software and logistic regression.

RESULTS

The *KRAS*, *NRAS*, *BRAF*, and *PIK3CA* mutations were detected in 44.6%, 3.4%, 3.7%, and 3.9% of CRC patients, respectively. *KRAS* mutations were more likely to occur in patients with moderate-to-high differentiation. *BRAF* mutations were more likely to occur in patients with right-sided CRC, poorly differentiated, or no

perineural invasion. Deficient MMR (dMMR) was detected in 7.9% of all patients and 16.8% of those with mucinous adenocarcinomas. *KRAS*, *NRAS*, *BRAF*, and *PIK3CA* mutations were detected in 29.6%, 1.1%, 8.1%, and 22.3% of patients with dMMR, respectively. The dMMR was more likely to occur in patients with a family history of CRC, aged < 50 years, right-sided CRC, poorly differentiated histology, no perineural invasion, and with carcinoma *in situ*, stage I, or stage II tumors.

CONCLUSION

This study analyzed the molecular profiles of *KRAS*, *NRAS*, *BRAF*, *PIK3CA*, and MMR/MSI in CRC, identifying key influencing factors, with implications for clinical management of CRC.

Key Words: Colorectal cancer; Deficient mismatch repair; Microsatellite instability; Gene mutation; Comprehensive analysis

Core Tip: This study presents a comprehensive analysis of *KRAS, NRAS, BRAF, PIK3CA* mutations, and mismatch repair (MMR)/microsatellite instability status in 2271 Chinese patients with colorectal cancer (CRC). Key findings include mutation frequencies, their association with clinicopathological characteristics, and the influence of MMR deficiency. These findings have significant implications for the clinical management of CRC, providing a foundation for personalized therapy and precision medicine in CRC.

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INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed cancer worldwide and the second leading cause of cancer-related mortality[1]. In China, CRC ranks fifth in terms of both incidence and mortality among all malignant tumors, according to the China Cancer Statistics report in 2018[2]. Advances in understanding the pathogenesis of CRC and in gene detection technologies have led to the identification of an increasing number of genes associated with CRC development and treatment. Notably, guidelines and expert consensus worldwide now recommend the detection of CRC-related genes such as microsatellite instability (MSI)/mismatch repair (MMR), *KRAS*, *NRAS*, and *BRAF*[3]. Furthermore, there is a growing interest among clinical investigators in exploring the clinical significance of other genes, such as *HER2*, *PIK3CA*, and *NTRK*, which have shown potential implications in the management of CRC.

According to relevant studies, approximately 40%-50% of CRC patients exhibit *KRAS* mutations, whereas *NRAS* mutations account for 3.8% of CRC cases. Patients with *KRAS* or *NRAS* mutations tend to exhibit poorer prognoses than those of patients with wild-type *KRAS* or *NRAS*, and they do not benefit from *EGFR* inhibitors[4,5]. The frequency of *BRAF* mutations in Asian CRC patients ranges from 5.4% to 6.7%[6]. In CRC, *BRAF V600E* mutations are associated with reduced overall survival, poor treatment response, and distinct patterns of metastasis and diffusion compared with those in wild-type *BRAF* tumors. Importantly, *BRAF* mutations are mutually exclusive of *KRAS/NRAS* mutations[4,7]. Additionally, *PIK3CA* mutations occur in a subset of the Chinese population, with a mutation rate of approximately 3.5%. *PIK3CA* mutations coexist with *RAS* mutations, unlike *BRAF* mutations. Furthermore, studies have suggested that *PIK3CA* mutations may serve as predictive markers for aspirin therapy[8]. In addition, approximately 10%-15% of CRC patients exhibit high MSI (MSI-H) or deficient MMR (dMMR). MSI-H is a crucial molecular pathway involved in CRC development. Early-stage CRC with the MSI-H/dMMR phenotype is associated with a more favorable prognosis, whereas MSI-H/dMMR is considered a poor prognostic factor for advanced CRC[9]. Notably, dMMR/MSI-H has emerged as a biomarker for predicting response to immune checkpoint blockade, representing a significant advancement in the treatment of patients with metastatic CRC who do not benefit from fluorouracil-based adjuvant chemotherapy[10-12].

Given the clinical implications of the MSI/dMMR, *KRAS*, *NRAS*, *BRAF*, and *PIK3CA* status, it is crucial for clinicians to accurately identify these molecular characteristics in patients with CRC to facilitate appropriate management decisions for patients and their at-risk family members. Therefore, this study aimed to evaluate the relationship between clinicopathological features and the prevalence of MMR/MSI, *KRAS*, *NRAS*, *BRAF*, and *PIK3CA* mutations, thereby enhancing our understanding of the clinical relevance of these genes in guiding treatment decisions.

MATERIALS AND METHODS

Patients

We conducted a retrospective analysis of the pathology reports from consecutively treated patients with CRC who underwent surgical resection at the China-Japan Friendship Hospital between January 2015 and December 2020. A total of 2271 eligible patients with CRC were included in this study. The clinical stage of each case was reassessed using the 8th edition of the American Joint Committee on Cancer Tumor Node Metastasis staging system. Detailed data on clinicopathological characteristics of the patients were collected and analyzed. This study was conducted in accordance with the ethical guidelines and regulations approved by the Ethics Committee of China-Japan Friendship Hospital (Approval No. QX2021-001-01). Given the retrospective nature of this study, the requirement for written informed consent was waived.

Immunohistochemical analysis

For each case, 4 μm thick sections were obtained from a representative block of formalin-fixed, paraffin-embedded tumor tissue. Immunohistochemistry (IHC) was performed using monoclonal antibodies against MLH1 (clone ES05, 1:20), MSH2 (clone FE11, 1:20), MSH6 (clone EP49, 1:20), and PMS2 (clone EPR3947, 1:20) sourced from Beijing Zhongshan Golden Bridge Biotechnology, China. IHC staining was carried out using an automated slide stainer (Ventana Benchmark) in combination with an OptiView DAB IHC Detection Kit (Ventana Medical Systems, AZ, United States). Evaluation of protein expression relied on the absence of nuclear staining in tumor cells or very faint nuclear staining in focal tumor cells, indicating a loss of protein expression (abnormal staining). The positive internal controls consisted of stromal/lymphoid cells and the nearby normal glandular epithelium of the bowel. To maintain consistency and accuracy, two specialized pathologists assessed the IHC results, and only samples with concordant interpretations were included in the present study.

KRAS/NRAS/BRAF/PIK3CA mutation testing

Genomic DNA was extracted from formalin-fixed and paraffin-embedded (FFPE) tissues, and subsequent quantitative polymerase chain reaction (qPCR) analysis was conducted using the human *KRAS*/*NRAS*/*PIK3CA*/*BRAF* mutation detection kit according to the manufacturer's instructions (AmoyDx, Xiamen, China). Briefly, a total volume of 25 μL was prepared, including a complex mixture comprising 0.3 μM primers and Taqman probes, 200 μM deoxynucleotide triphosphates, 200 μM Taq polymerase, and 90 ng of DNA. qPCR amplification was performed carried out using the ABI 7500 instrument (Thermo Fisher Scientific, Massachusetts, United States) according to the manufacturer's instructions. The resulting data were analyzed using 7500 software ver 2.3 (Applied Biosystems, Foster City, CA, United States).

MSI test

CRC tissue samples and adjacent normal tissue samples were collected from patients. A pathologist reviewed hematoxylin and eosin-stained slides of the tumors, and areas containing < 10% viable tumor cells were marked for macrodissection to enrich the tumor DNA. Genomic DNA was then extracted from all paired tumor-normal samples using the AmoyDx® FFPE DNA Kit (Xiamen, China). Microsatellite status was determined using six microsatellite markers (*NR21*, *NR24*, *NR27*, *BAT25*, *BAT26*, and *MONO-27*) and was detected using an MSI detection kit (Microread, Beijing, China). The reaction complex was subsequently analyzed by DNA fragmentation assays using an Applied Biosystems 3500DX instrument (Massachusetts, United States). Further details on the specific practices for MSI detection can be found in references[13]. Allelic sizes were evaluated using GeneMapper software ver 4.1 (Thermo Fisher Scientific, Waltham, MA, United States). MSI status was classified as microsatellite stable (MSS), MSI-low (MSI-L, with instability in one marker), and MSI-H (with instability in more than two markers).

Statistical analysis

Statistical analyses were conducted using SPSS (version 22; SPSS Inc., Chicago, IL, United States) or Prism software (version 7; San Diego, CA, United States). Data that did not meet the criteria for the χ^2 test were combined into appropriate groups to meet the required standards. Logistic regression analysis was used to determine the factors influencing driver gene mutations. P values < 0.05 were considered statistically significant.

RESULTS

Characteristics of the patients

A total of 2271 patients diagnosed with CRC were included in the analysis. Among these patients, 1361 (59.9%) were male and 910 (40.1%) were female. Of the total number of patients, 1983 (87.3%) were over 50 years of age, 300 (13.2%) had a family history of CRC, 1762 (77.6%) had no history of smoking, and 1848 (81.4%) had no history of alcohol consumption. Histologically, 2150 (99.1%) patients had adenocarcinomas, including 113 (5.0%) diagnosed with mucinous adenocarcinomas. Tumor grading, according to the World Health Organization (WHO) criteria (Fourth Edition of the WHO Classification of Tumors of the Digestive System), revealed that 226 (10.0%) tumors were poorly differentiated, while 1878 (82.7%) were moderately or well differentiated. Most tumors were in stages II and III (36.8% and 41.2%, respectively). Right-sided colon cancer was diagnosed in 551 (24.3%) patients, while left-sided colon cancer was diagnosed in 1718 (75.6%) patients. Additionally, 489 (21.5%), 786 (34.6%), and 486 (21.4%) patients had cancerous nodules, perineural invasion, and vascular invasion, respectively. Detailed clinicopathological characteristics of the patients are summarized

in Table 1.

KRAS/NRAS/BRAF/PIK3CA mutation characteristics and affecting factors

The status of *KRAS*, *NRAS*, *BRAF*, and *PIK3CA* mutations in 2271 Chinese patients with CRC is presented in Figure 1 and Table 2. *KRAS* mutations were detected in 44.6% (1014/2271) of all patients and 44.2% (50/113) of patients with mucinous adenocarcinomas. Among the overall patient population, 28.8% (653/2271) of the *KRAS* mutations occurred in exon 2, 1.5% (34/2271) in exon 3, 2.1% (48/2271) in exon 4, and 0.2% (6/2271) were indistinguishable between exons 2/3 or 2/4. In patients with mucinous adenocarcinoma, 37.2% (42/113), 2.7% (3/113), and 4.4% (5/113) of the mutations were in exons 2, 3, and 4, respectively. Of the analyzed samples, 3.4% (78/2271) harbored *NRAS* mutations, of which 1.5% (33/2271) occurred in exon 2 and 1.1% (25/2271) in exon 3. No *NRAS* mutations were detected in patients with mucinous adenocarcinoma. Interestingly, six patients displayed concurrent mutations in both *KRAS* and *NRAS*. *PIK3CA* mutations were detected in 88 patients (3.9%, 88/2271), of whom 43 (48.8%, 43/88) had *KRAS* mutations as well. *PIK3CA* mutations were present in 43 of 1014 (4.2%) patients with *KRAS* mutations, compared to 45 of 1256 (3.6%) patients with wild-type *KRAS*. Notably, in mucinous adenocarcinoma, *PIK3CA* exon 20 mutations accounted for 7.1% (8/113) of the cases. Furthermore, *BRAF V600E* mutations were detected in 85 of the 2271 patients with CRC (3.7%) and in 14 of the 113 patients with mucinous adenocarcinoma (12.4%). The mutual exclusivity of *KRAS* (exons 2, 3, and 4), *NRAS* (exons 2, 3, and 4), and *BRAF* mutations was confirmed, as none of the patients with *KRAS* or *NRAS* mutations displayed simultaneous *BRAF* mutations. However, three tumors exhibited concurrent mutations in *BRAF* and *PIK3CA*.

In addition, we investigated the factors affecting the mutation status of *KRAS*, *NRAS*, *BRAF*, and *PIK3CA* (Supplementary Table 1, Figure 2). Logistic regression analysis revealed that *KRAS* mutations were more prevalent in patients with moderately and highly differentiated tumors ($P = 0.003$). *BRAF* mutations were more frequently observed in patients with right-sided CRC, poorly differentiated tumors, and no perineural invasion ($P < 0.01$). Additionally, *PIK3CA* mutations were more common in patients with right-sided CRC ($P = 0.004$). No statistically significant differences were observed between patients with wild-type *NRAS* and those with mutations.

Characteristics of dMMR and MSI and influencing factors

All 2271 patients with CRC included in our study underwent both MMR-IHC and MSI-PCR analyses. Abnormal MMR IHC results were detected in 179 tumors (7.9% of patients). Specifically, 2, 3, 16, and 28 patients exhibited loss of MLH1, MSH2, MSH6, and PMS2 expression, respectively. Furthermore, 96, 28, and 6 patients showed simultaneous loss of MLH1/PMS2, MSH2/MSH6, and MLH1/PMS2/MSH6 expression, respectively. In terms of MSI status, 186 patients (8.2% of the total) were classified as having MSI, including seven patients (3.8%) with MSI-L and 179 patients (96.2%) with MSI-H. The concordance between MSI-H cases and those exhibiting abnormal MMR IHC was remarkably high, with 95.5% of cases with abnormal MMR IHC showing the MSI-H status. However, eight patients (0.4%) with MSI-H showed no abnormalities in MMR IHC, and six patients (3.4%) with abnormal MMR IHC were found to be MSS upon initial assessment (Table 3).

The relationships between clinicopathological features and MSI or MMR status are shown in Supplementary Table 2. The incidence of dMMR in mucinous adenocarcinomas was as high as 16.8%. Interestingly, the prevalence of *KRAS* mutations in patients with dMMR was 29.6% (53 out of 179), which was lower than that observed in the general population. Additionally, the frequency of *BRAF* mutations among patients with dMMR was 22.3% (40 out of 179), as depicted in Figure 3.

We conducted additional analyses to assess the clinicopathological factors associated with dMMR. Our findings revealed that dMMR was more likely to accumulate in patients aged < 50 years, with a positive family history, and with tumors located in the right colorectal site ($P < 0.001$). In addition, dMMR tumors were significantly associated with poorly differentiated histology ($P < 0.001$) and the absence of perineural invasion ($P < 0.001$), particularly in stage carcinoma *in situ* (Tis; $P = 0.007$) or stage II ($P = 0.001$), as illustrated in Figure 4.

DISCUSSION

Mutations in *KRAS*, *NRAS*, *BRAF*, and *PIK3CA* and the assessment of MMR/MSI status play crucial roles in evaluating patients with CRC. In this real-world study, we conducted a retrospective analysis of *KRAS*, *NRAS*, *BRAF*, and *PIK3CA* mutated genes, as well as MMR protein status, in a cohort of 2271 patients with CRC from northern China. The analysis of this large-scale dataset aimed to elucidate the clinicopathological characteristics and clinical relevance of these significant driver genes.

The mutation rates and profiles of *KRAS*, *NRAS*, *BRAF*, and *PIK3CA* in our study were consistent with those of previous reports[14,15]. Mucinous colorectal adenocarcinoma, although a rare histological type accounting for 5%-15% of CRC cases, is associated with worse survival than conventional adenocarcinoma[16,17]. Furthermore, mucinous adenocarcinoma exhibits higher frequencies of *KRAS*, *BRAF*, and *PIK3CA* mutations when compared to those in all CRC types[18]. In our study, we observed mutation rates of 60.2%, 12.4%, and 7.1% for *KRAS*, *BRAF*, and *PIK3CA*, respectively, in mucinous adenocarcinoma, compared to 44.6%, 3.7%, and 3.9%, respectively, in all CRC cases. These findings are consistent with those reported by Li *et al*[16].

To provide meaningful insights for clinical practice, we analyzed the clinicopathological factors associated with driver gene mutations. Our multivariate logistic regression analysis revealed that tumor differentiation grade was a key factor related to *KRAS* mutations. Specifically, patients with moderately to highly differentiated tumors were more likely to harbor *KRAS* mutations, as reported by Imamura *et al*[19]. Interestingly, no significant clinicopathological differences

Table 1 Clinicopathological characteristics of patients cohort, *n* (%)

Characteristics	All cases (*n* = 2271)	Characteristics	All cases (*n* = 2271)
Sex		Perineural invasion	
Male	1361 (59.9)	No	1484 (65.3)
Female	910 (40.1)	Yes	786 (34.6)
Age category (yr)		NA	1 (< 0.01)
≤ 50	288 (12.7)	Vascular invasion	
> 50	1983 (87.3)	No	1779 (78.3)
Smoking status		Yes	486 (21.4)
No	1762 (77.6)	NA	6 (0.3)
Yes	509 (22.4)	Histological type	
Drinking status		Adenocarcinoma	2250 (99.1)
No	1848 (81.4)	Mucinous adenocarcinoma	113 (5.0)
Yes	423 (18.6)	Others	21 (0.9)
Family history		Histological differentiation grade	
No	1971 (86.8)	Poorly differentiated	226 (10.0)
Yes	300 (13.2)	Moderately/well differentiated	1878 (82.7)
Tumor location		NA	167 (7.4)
Right colon	551 (24.3)	Stage	
Left colon	1718 (75.6)	Tis	18 (0.8)
Liver	2 (0.1)	I	304 (13.4)
Cancer nodules		II	836 (36.8)
No	1782 (78.5)	III	935 (41.2)
Yes	489 (21.5)	IV	178 (7.8)

Tis: Carcinoma *in situ*. NA: Not available.

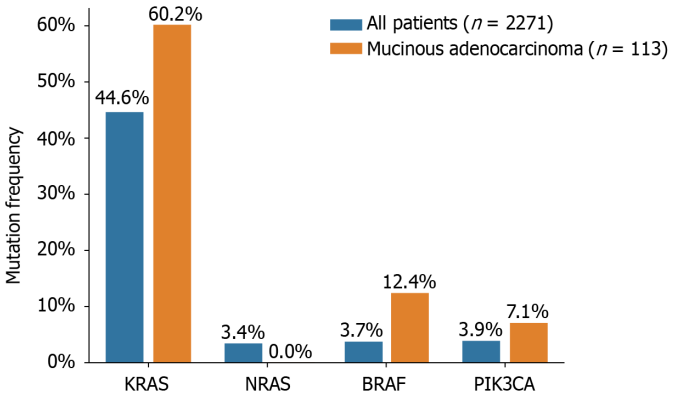
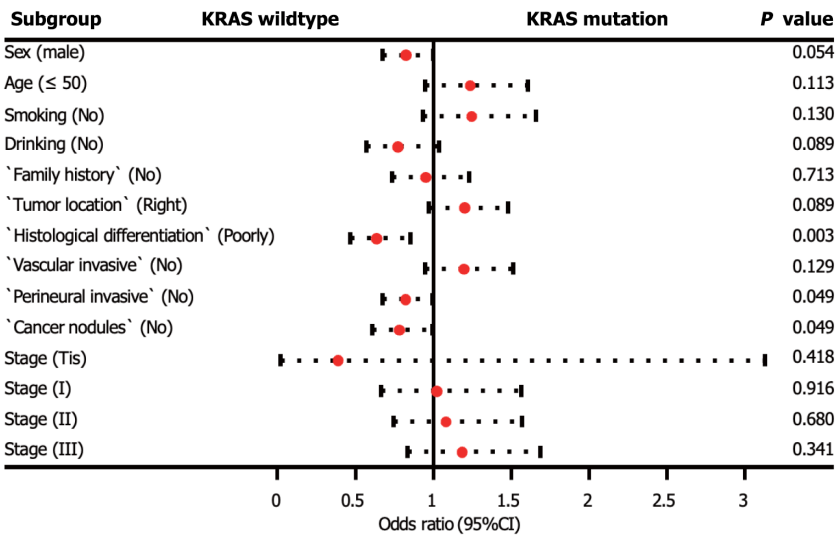


Figure 1 *KRAS*/*NRAS*/*BRAF*/*PIK3CA* mutation frequency.

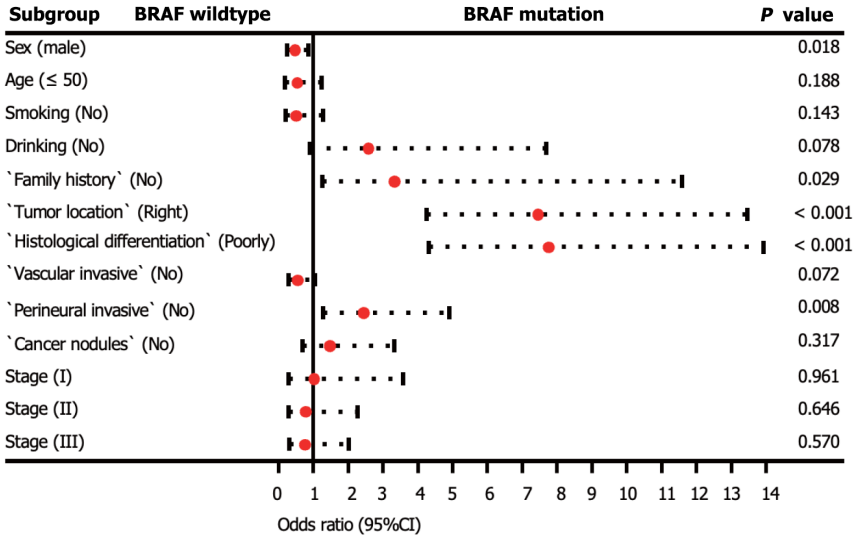
were found between *NRAS* mutated tumors and wild-type tumors in our study, as well as in other studies. Additionally, we found that high *BRAF* mutation rates were associated with low differentiation, the absence of perineural invasion, and right-sided colon cancer, which is consistent with the results of previous research[20,21]. Moreover, our study identified cancer location as the only relevant factor for *PIK3CA* mutations, with these mutations being enriched in right-sided CRC.

MSI is a molecular manifestation of a defective MMR system[22]. PCR-based MSI detection is commonly employed because it can provide insight into the status of the MMR system and exhibits high concordance with MMR protein detection through immunohistochemical analysis. Multiple clinical trials have demonstrated a strong correlation between

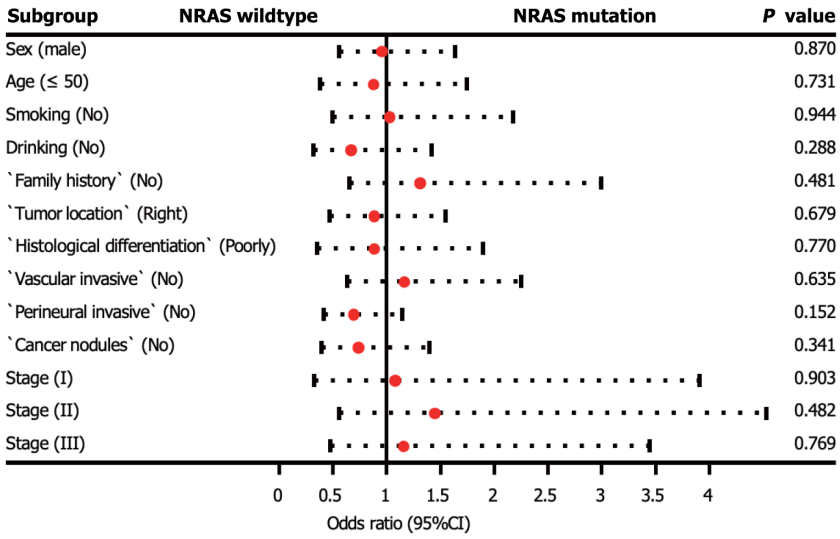
A


Subgroup
KRAS wildtype
KRAS mutation
P value
Sex (male) 0.054
Age (≤ 50) 0.113
Smoking (No) 0.130
Drinking (No) 0.089
`Family history` (No) 0.713
`Tumor location` (Right) 0.089
`Histological differentiation` (Poorly) 0.003
`Vascular invasive` (No) 0.129
`Perineural invasive` (No) 0.049
`Cancer nodules` (No) 0.049
Stage (Tis) 0.418
Stage (I) 0.916
Stage (II) 0.680
Stage (III) 0.341
0 0.5 1 1.5 2 2.5 3
Odds ratio (95%CI)

B


Subgroup
BRAF wildtype
BRAF mutation
P value
Sex (male) 0.018
Age (≤ 50) 0.188
Smoking (No) 0.143
Drinking (No) 0.078
`Family history` (No) 0.029
`Tumor location` (Right) < 0.001
`Histological differentiation` (Poorly) < 0.001
`Vascular invasive` (No) 0.072
`Perineural invasive` (No) 0.008
`Cancer nodules` (No) 0.317
Stage (I) 0.961
Stage (II) 0.646
Stage (III) 0.570
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Odds ratio (95%CI)

C


Subgroup
NRAS wildtype
NRAS mutation
P value
Sex (male) 0.870
Age (≤ 50) 0.731
Smoking (No) 0.944
Drinking (No) 0.288
`Family history` (No) 0.481
`Tumor location` (Right) 0.679
`Histological differentiation` (Poorly) 0.770
`Vascular invasive` (No) 0.635
`Perineural invasive` (No) 0.152
`Cancer nodules` (No) 0.341
Stage (I) 0.903
Stage (II) 0.482
Stage (III) 0.769
0 0.5 1 1.5 2 2.5 3 3.5 4
Odds ratio (95%CI)

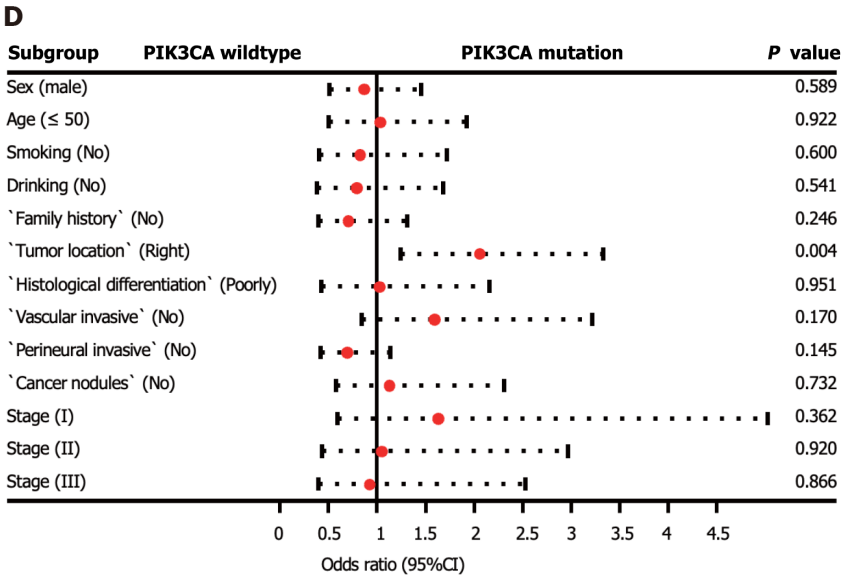


Figure 2 Multivariate logistic regression analyses for *KRAS*, *BRAF*, *NRAS*, and *PIK3CA* mutation detection. A: *KRAS* mutation detection; B: *BRAF* mutation detection; C: *NRAS* mutation detection; D: *PIK3CA* mutation detection. If the odds ratio is less than 1, then the predictor is associated with *KRAS*, *BRAF*, *NRAS*, and *PIK3CA* mutation detection. OR: Odds ratio; CI: Confidence interval.

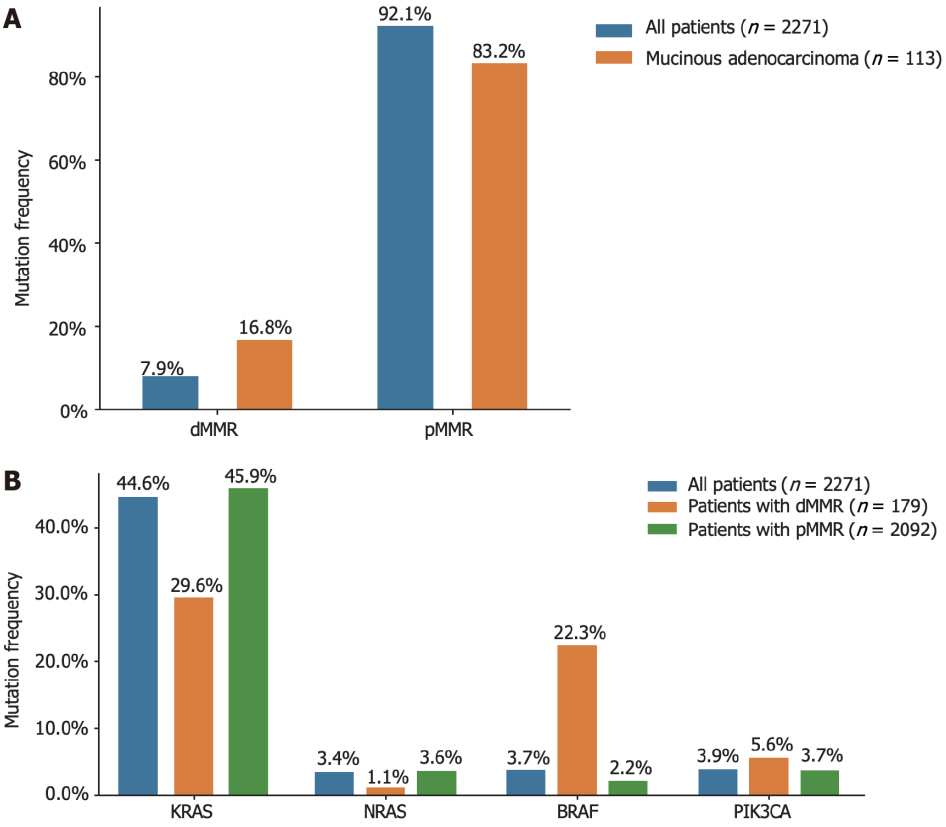


Figure 3 The mismatch repair deficiency status distributed across different histological types and driver genes. A: Different histological types; B: Different driver genes. dMMR: Deficient mismatch repair; pMMR: Proficient mismatch repair.

molecular MSI and protein-based MMR detection[23]. In the present study, the consistency between MSI-PCR and MMR-IHC detection was 99.6%, which surpassed that reported in previous clinical studies. In addition, we found a higher incidence of dMMR in patients with mucinous adenocarcinoma (16.8%) than in all patients (7.9%), which is consistent with previous reports[18]. Among patients with dMMR, there was an enrichment of *BRAF* (22.3% *vs* 3.7% in all CRC) and *PIK3CA* mutations (5.6% *vs* 3.9% in all CRC), as well as a lower frequency of *KRAS* (29.6% *vs* 44.6% in all CRC) and *NRAS* mutations (1.1% *vs* 3.4% in all CRC). *BRAF V600E* has been strongly correlated with *MLH1* promoter methylation, leading to dMMR[24]. Additionally, *KRAS*/*NRAS* mutations have been shown to be mutually exclusive of dMMR[25]. In our

Table 2 Distribution of *KRAS*, *NRAS*, *PIK3CA*, and *BRAF* mutations in patients with colorectal carcinoma, *n* (%)

Mutation genes	All patients (*n* = 2271)	Mucinous adenocarcinoma (*n* = 113)
KRAS		
Exon 2	653 (28.8)	42 (37.2)
Exon 3	34 (1.5)	3 (2.7)
Exon 4	48 (2.1)	5 (4.4)
Exon 2/3	3 (0.1)	0
Exon 2/4	3 (0.1)	0
NRAS		
Exon 2	33 (1.5)	0
Exon 3	25 (1.1)	0
BRAF		
Exon 15	85 (3.7)	14 (12.4)
PIK3CA		
Exon 20	88 (3.9)	8 (7.1)

Table 3 Comparison of mismatch repair immunohistochemistry and microsatellite instability testing, *n* (%)

Test results of MSI	MMR IHC normal (*n* = 2092)	MMR IHC abnormal (*n* = 179)
MSS	2079 (99.4)	6 (3.4)
MSI-L	5 (0.2)	2 (1.1)
MSI-H	8 (0.4)	171 (95.5)

MSI: Microsatellite instability; MSI-H: Microsatellite instability-high; MSI-L: Microsatellite instability-low; MSS: Microsatellite-stable; MMR: Mismatch repair; IHC: Immunohistochemistry.

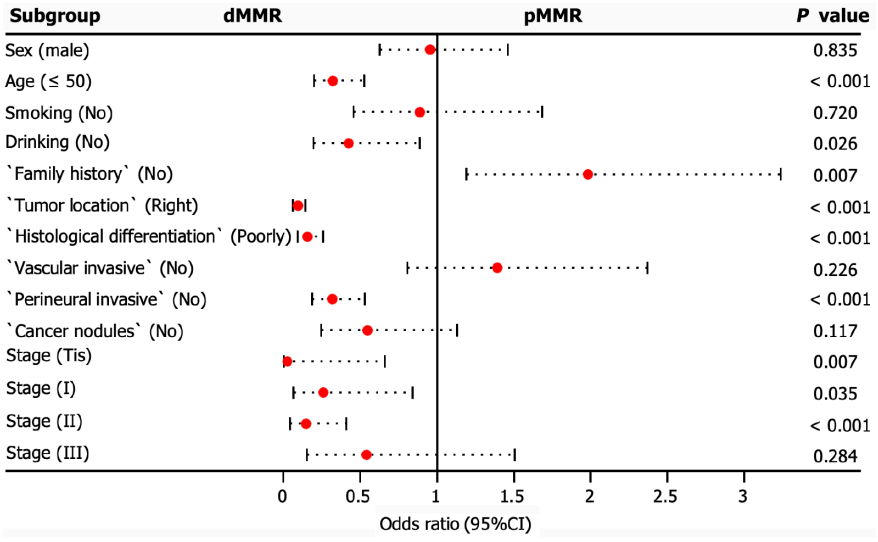


Figure 4 Multivariate logistic regression analyses for deficient mismatch repair detection. If the odds ratio is less than 1, then the predictor is associated with deficient mismatch repair detection. CI: Confidence interval; OR: Odds ratio; dMMR: Deficient mismatch repair; pMMR: Proficient mismatch repair; Tis: Carcinoma *in situ*.

study, factors associated with a higher incidence of dMMR included age (≤ 50 years), right-sided CRC, poorly differentiated histology, and early stages (Tis, I, and II), which were similar to the influencing factors reported by Li *et al*[26] and Guo *et al*[27]. Notably, our study demonstrated a trend of younger patients with CRC, which may have arisen from the smaller age stratification in our cohort. It has been reported that dMMR is a good prognostic indicator for stage II CRC and that patients do not benefit from 5-fluorouracil-based adjuvant chemotherapy[28]. In this study, we collected clinical information from 15 patients who experienced relapse following surgery at stage II, with 12 patients exhibiting the proficient MMR (pMMR) status and experiencing a disease-free survival of less than 30 months. Limited data indicate that patients with pMMR stage II are prone to relapse, necessitating the collection of additional patient data for a more comprehensive comparative analysis during follow-up.

CONCLUSION

We conducted a comprehensive analysis of the clinicopathological characteristics and factors influencing *KRAS*, *NRAS*, *BRAF*, *PIK3CA*, and MMR/MSI in CRC. The results revealed that patients with mucinous adenocarcinoma exhibited higher mutation rates in *KRAS*, *BRAF*, *PIK3CA*, and dMMR. Further validation is required to confirm the clinicopathological features and prognostic value of these markers, particularly in relation to different treatment regimens. Nonetheless, the findings obtained from this large-scale, real-world dataset undoubtedly contribute to a better understanding of the significance of genetic testing and provide valuable guidance for clinical practice.

FOOTNOTES

Author contributions: Jie JZ and Zhong DR conceived and coordinated the research; Chen H, Jiang RY, Wang Y, Feng QQ, Luo J, and Ning W collected data from patients; Chen H, Hua Z, and Wang XW analyzed data and wrote the manuscript; Shi XL, Ning W, Shi YF, Zhang DK, and Wang B revised the manuscript; and all authors read and approved the final version of the manuscript.

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