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Effects of KRAS/NRAS/BRAF Mutations on the Clinicopathological Features of TP53-Mutant Colorectal Cancer

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Effects of KRAS/NRAS/BRAF Mutations on the Clinicopathological Features of TP53-Mutant Colorectal Cancer

Shoujia Zhang Ge Liu

Abstract Objective The present study was designed to evaluate the impact of KRAS/NRAS/BRAF mutations on the clinicopathological features of TP53-mutant colorectal cancer.**Methods** Clinicopathological data from 113 patients who underwent radical surgery for colorectal cancer at the Department of Colorectal Surgery, the First Affiliated Hospital of Dalian Medical University, between September 2023 and April 2026, were retrospectively collected. The data included sex, age, preoperative CEA level, operative time, tumor location, tumor size, pathological type, histological type, lymph node metastasis, tumor deposit metastasis,intravascular tumor thrombus, perineural invasion, T stage, N stage, M stage, postoperative pathological stage, and relevant gene status.Patients were divided into an experimental group (TP53-mutant) and a control group (non-TP53-mutant) based on TP53 mutation status.Based on KRAS/NRAS/BRAF status (wild type or mutant), each of the two groups was further divided into two subgroups, resulting in the following four groups: KRAS/NRAS/BRAF wild-type with TP53-mutant, KRAS/NRAS/BRAF mutant with TP53-mutant,KRAS/NRAS/BRAF wild-type with non-TP53-mutant,and KRAS/NRAS/BRAF mutant with non-TP53-mutant.The chi-square test was used to compare the differences in clinicopathological features among the four groups, and a p-value $\leq$ 0.05 was considered statistically significant.**Results** Among the 113 patients with colorectal cancer, the mutation frequencies of TP53, KRAS, NRAS, and BRAF genes were 73.5%, 38.1%, 4.4%, and 3.5%, respectively.Stratified analysis using the chi-square test and the chi-square partition method revealed that KRAS/NRAS/BRAF mutations significantly influenced age ($p = 0.008$), preoperative CEA level ($p = 0.022$), distant metastasis (M stage, $p = 0.001$), and TNM stage (stage grouping, $p = 0.001$) in patients with TP53-mutant colorectal cancer. In contrast, for patients with non-TP53-mutant colorectal cancer, KRAS/NRAS/BRAF mutations had no significant impact on clinicopathological features ($p > 0.05$).**Conclusion** In TP53-mutant colorectal cancer, mutations in KRAS, NRAS, or BRAF can impact the postoperative pathological stage, and this effect confers prognostic significance for the affected patient subgroup.

Keywords： Colorectal cancer KRAS NRAS BRAF TP53

Colorectal cancer (CRC) is the third most common malignancy globally and a major cause of cancer-related mortality[1]. Currently, colorectal cancer is treated with a comprehensive strategy centered on surgical resection, including chemotherapy, targeted therapy, immunotherapy, and radiotherapy[2–4]. With the in-depth investigation into the pathogenesis of colorectal cancer and the rapid development of molecular targeted therapy techniques, appropriate detection of relevant molecular markers has become a critical component in the clinical diagnosis and treatment of this disease[2–5]. Among these, KRAS, NRAS, BRAF, TP53, APC, and PIK3CA are the most frequently mutated genes in colorectal cancer[6]. The mutation status of these genes is closely associated with postoperative pathological staging and prognosis of colorectal cancer patients[7–10]. Based on the gene mutation profile of individual

patients, corresponding targeted therapy or immunotherapy agents can be selected[6]. Meanwhile, chemotherapy regimens are determined in combination with histopathological findings and TNM staging, thereby establishing a rational individualized treatment strategy[3, 4]. This study retrospectively collected clinicopathological data from patients who underwent surgical treatment for colorectal cancer and concurrent genetic testing in the Department of Colorectal Surgery, The First Affiliated Hospital of Dalian Medical University, from September 2023 to April 2026. The chi-square test was used for statistical analysis. The aim was to investigate the impact of KRAS/NRAS/BRAF mutations on the clinicopathological features of patients with TP53-mutant colorectal cancer.

1 Materials and Methods：

1.1 General Information：

Participants： A total of 113 patients who underwent radical surgery for colorectal cancer at the Department of Colorectal Surgery, the First Affiliated Hospital of Dalian Medical University, from September 2023 to April 2026 were retrospectively enrolled.

Inclusion criteria were as follows: (1) patients aged ≥ 18 years who were diagnosed with colorectal cancer and underwent surgical treatment; (2) patients who underwent genetic testing after surgery.

Exclusion criteria were as follows: (1) patients with incomplete clinicopathological data; (2) patients with severe cardiac or pulmonary dysfunction.

1.2 Data Collection：

Clinical and pathological data and genetic testing results were retrospectively collected from 113 patients who underwent radical surgery for colorectal cancer at the Department of Colorectal Surgery, the First Affiliated Hospital of Dalian Medical University, from September 2023 to April 2026. The variables collected included sex, age, preoperative carcinoembryonic antigen (CEA) level, operative time, tumor location, tumor size, histological type, histologic grade, lymph node metastasis, tumor deposit metastasis, venous invasion, perineural invasion, T category, N category, M category, postoperative pathological stage, and relevant genetic testing results.

1.3 Statistical Analysis：

All statistical analyses were performed using SPSS version 29.0 (IBM Corp., Armonk, NY, USA). Patients were initially grouped by TP53 mutation status (TP53-mutant vs. non-TP53-mutant), and then further stratified by KRAS/NRAS/BRAF status (wild-type vs. mutant), yielding four subgroups(Figure1): (1) KRAS/NRAS/BRAF wild-type with TP53-mutant; (2) KRAS/NRAS/BRAF mutant with TP53-mutant; (3) KRAS/NRAS/BRAF wild-type with non-TP53-mutant; and (4) KRAS/NRAS/BRAF mutant with non-TP53-mutant.Clinicopathological differences among the four groups were compared using the chi-square test, with a two-sided $P < 0.05$ considered statistically significant.

2 Results

In this cohort, the mutation rates of TP53, KRAS, NRAS, and BRAF were 73.5%, 38.1%, 4.4%, and 3.5%, respectively. TP53 exhibited the highest mutation rate

among the four genes(Table1).Patients were divided into TP53-mutant and non-TP53-mutant groups according to TP53 mutation status, and then further stratified by KRAS/NRAS/BRAF status (wild-type vs. mutant) into four subgroups(Figure1): (1) KRAS/NRAS/BRAF wild-type with TP53 mutant; (2) KRAS/NRAS/BRAF mutant with TP53 mutant; (3) KRAS/NRAS/BRAF wild-type with non-TP53-mutant;and (4) KRAS/NRAS/BRAF mutant with non-TP53-mutant.The chi-square analysis revealed that KRAS/NRAS/BRAF mutation status was significantly associated with age ($P = 0.037$), preoperative CEA level ($P = 0.019$), histologic grade ($P = 0.007$), M category ($P = 0.001$), and TNM stage ($P = 0.006$)(Table 2).The clinicopathological variables with $P < 0.05$ were further analyzed using the chi-square partitioning method. In the TP53-mutant group, KRAS/NRAS/BRAF mutation status was significantly associated with age ($P = 0.008$), preoperative CEA level ($P = 0.022$), M category ($P = 0.001$), and TNM stage ($P = 0.001$). In contrast, no significant associations were observed between KRAS/NRAS/BRAF mutation status and clinicopathological characteristics in the non-TP53-mutant group (all $P > 0.05$) (Table 3).

3 Discussion

Treatment strategies for colorectal cancer are primarily determined by the histopathological characteristics and anatomical extent of the tumor. Among these, postoperative pathological stage serves as a critical indicator for determining the need for adjuvant therapy[3, 4]. With the development of precision oncology and continuous advances in molecular sequencing technologies, research into the molecular mechanisms of colorectal cancer has been increasingly refined, and certain genetic mutations have been identified as influencing tumorigenesis, disease progression, and prognosis[3, 4, 11]. The occurrence of gene mutations in colorectal cancer is largely attributable to two primary mechanisms: inactivation of tumor suppressor genes and activation of oncogenes. The tumor suppressor genes most frequently implicated include APC, TP53, and FBXW7, whereas the oncogenes predominantly involved comprise KRAS, NRAS, BRAF, and PIK3CA[6]. The p53 protein, encoded by the TP53 gene, is a potent tumor suppressor that prevents abnormal cell growth and proliferation by regulating cell division [12, 13]. It also plays a critical role in DNA repair and cell cycle regulation, and is thus often referred to as the “guardian of the genome” [14]. Mutations in this gene can arise at early stages of colorectal carcinogenesis, are detectable in the majority of adenomas and carcinomas, and exhibit an overall mutation rate of approximately 50–60% [6]. The KRAS gene is involved in the regulation of cell division. Its mutations lead to constitutive activation of the MAPK/ERK and PI3K/AKT signaling pathways. These mutations are relatively common, occurring at a frequency of 30%–40%[15, 16], and have a substantial impact on patient prognosis and therapeutic decision-making [6]. Notably, KRAS mutations also confer resistance to anti-EGFR antibodies [17].NRAS, another member of the RAS gene family alongside KRAS, encodes a protein involved in the regulation of cell division. Although mutations in this gene are relatively infrequent, occurring in approximately 3%–5% of cases, they are closely associated with poor prognosis and

tumor metastasis [6]. The BRAF gene encodes a serine/threonine kinase that belongs to the RAS/MAPK signaling pathway and participates in the regulation of cell proliferation, differentiation, migration, and apoptosis [18, 19].BRAF mutations are relatively uncommon, with a frequency of approximately 8%–10% [20]; they are predominantly observed in right-sided colon cancers and are associated with MSI-H tumors[6]. The most common type of BRAF alteration is the V600E mutation [21], which serves as a marker of tumor aggressiveness [22]. Patients harboring this mutation generally do not derive benefit from anti-EGFR therapy [21, 23, 24].

In the present study, the mutation rates were 73.5% for TP53, 38.1% for KRAS, 4.4% for NRAS, and 3.5% for BRAF. TP53 is a tumor suppressor gene that can undergo mutation at early stages of tumorigenesis, exhibits a high mutation frequency, and is often referred to as the “guardian of the genome”. In contrast, KRAS, NRAS, and BRAF are proto-oncogenes. On this basis, we first stratified patients according to TP53 mutation status, and then further stratified them based on KRAS/NRAS/BRAF mutation status(mutant vs. wild-type). Statistical analyses were performed using the chi-square test.

Our results showed that in TP53-mutant colorectal cancer, mutations in KRAS/NRAS/BRAF were significantly associated with patient age ($p = 0.008$), preoperative CEA level ($p = 0.022$), distant metastasis ($p = 0.001$), and TNM stage ($p = 0.001$), suggesting that when these proto-oncogenes are concurrently mutated with TP53, patients present with more advanced pathological stages. In contrast, in non-TP53-mutant colorectal cancer, mutations in KRAS/NRAS/BRAF showed no significant associations with any postoperative clinicopathological features (all $p > 0.05$), indicating that concurrent mutations of these genes may not affect pathological staging in patients without TP53 mutations. Previous studies have also demonstrated that patients with concurrent mutations in KRAS/NRAS/BRAF and TP53 have a poor prognosis[6].

4 Conclusion

In TP53-mutant colorectal cancer, KRAS/NRAS/BRAF mutations are associated with postoperative pathological stage and prognosis, suggesting that they influence prognosis through their impact on pathological staging.

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Table 1. Gene mutation rates

gene mutation	cases	Mutation rate (%)
KRAS gene	113	
mutant	43	38.1
wild-type	70	61.9
NRAS gene	113	
mutant	5	4.4
wild-type	108	95.6
BRAFgene	113	
mutant	4	3.5

wild-type	109	96.5
TP53gene	113	
mutant	83	73.5
non-mutant	30	26.5

		TP53-mutant	non-TP53-mutant

Table 2. Comparison of clinicopathological characteristics among the four groups

Table 3. Comparison of clinicopathological characteristics between two groups using the chi-square partition method.

Clinicopathological characteristics	Cases	TP53-mutant		non-TP53-mutant		$\chi2$ value	P value
		KRAS/ NRAS/ BRAF Wild-type	KRAS/ NRAS/ BRAF mutant	KRAS/ NRAS/ BRAF Wild-type	KRAS/ NRAS/ BRAF mutant		
sex							
male	72	34	17	10	11		
female	41	15	17	3	6	4.437	0.221
age							
≤60	41	22	6	7	6		
>60	72	27	28	6	11	8.422	0.037
Preoperative CEA level							
≤5ng	77	41	20	7	9		
>5ng	36	8	14	6	8	9.838	0.019
tumor location							
Right-sided colon	28	8	8	4	8		
Left-sided colon	26	14	4	4	4		
Rectum	51	24	20	4	3		
Multiple primary cancer	8	3	2	1	2	13.576	0.136
tumor size							
≤5	63	30	19	7	7		
>5	50	19	15	6	10	2.078	0.575
histological type							
Protruded type	22	13	5	1	3		
Ulcerative type	90	35	29	12	14		
infiltrative type	1	1	0	0	0	4.741	0.573
histologic grade							
Well and moderately differentiated	64	32	18	9	5		
Moderately and poorly differentiated	37	13	15	3	6		
Poorly differentiated	12	4	1	1	6	17.664	0.007
lymph node metastasis							
Lymph node-negative	53	21	14	8	10		
Lymph node-positive	60	28	20	5	7	2.858	0.422
tumor deposit metastasis							
Tumor deposit-negative	86	40	22	12	12		
Tumor deposit-positive	27	9	12	1	5	5.414	0.144
venous invasion							
Yes	42	15	15	4	8		
No	71	34	19	9	9	2.545	0.471
perineural invasion							
Yes	73	30	27	6	10		
No	40	19	7	7	7	5.688	0.127
T category							
T1-T3	15	8	3	2	2		
T4	98	41	31	11	15	1.066	0.771
N category							
N0	48	20	11	8	9		
N1	37	16	16	1	4		
N2	28	13	7	4	4	7.963	0.246
M category							
M0	90	46	22	12	10		
M1	23	3	12	1	7	16.636	0.001
Stage							
I-II stage	10	5	1	2	2		
III stage	80	41	21	10	8		
IV stage	23	3	12	1	7	18.444	0.006

		KRAS/ NRAS/ BRAF Wild type	KRAS/ NRAS/ BRAF mutant	χ2 value	P value	KRAS/ NRAS/ BRAF Wild type	KRAS/ NRAS/ BRAF mutant	χ2 value	P value
age									
≤60	41	22	6			7	6		
>60	72	27	28	6.668	0.008	6	11	1.033	0.460
Preoperative CEA level									
≤5ng	77	41	20			7	9		
>5ng	36	8	14	6.363	0.022	6	8	0.002	1.000
histologic grade									
Well and moderately differentiated	64	32	18			9	5		
Moderately and poorly differentiated	37	13	15			3	6		
Poorly differentiated	12	4	1	3.258	0.234	1	6	5.275	0.094
M category									
M0	90	46	22			12	10		
M1	23	3	12	11.537	0.001	1	7	4.224	0.092
Stage									
I-II stage	10	5	1			2	2		
III stage	80	41	21			10	8		
IV stage	23	3	12	12.206	0.001	1	7	4.265	0.179

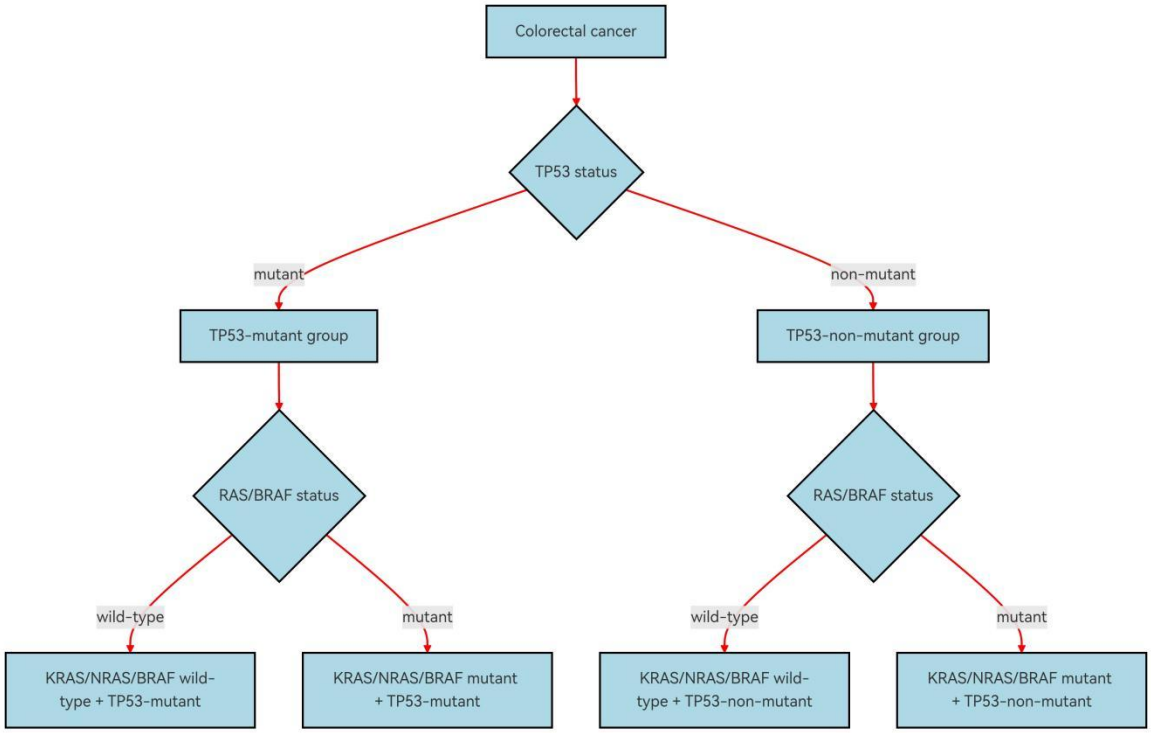


Figure 1. Flowchart of patient grouping in colorectal cancer.

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1.Data Availability Statement：

All data generated or analysed during this study are included in this published article [and its supplementary information files].

2.Ethical Approval and Accordance:

The study protocol was approved by the Ethics Committee of First Affiliated Hospital of Dalian Medical University for submitting a research paper for publication.The Ethics References No:PJ-KS-KY-2026-682.This study was performed in compliance with the Declaration of Helsinki.

3.Consent to Publish：

No identifying individual patient information, clinical photographs, or identifiable images of participants are presented in this manuscript. All included figures are pathological micrographs, statistical graphs and aggregated clinicopathological data without individual-level identifiable information. Hence, consent to publish is not applicable to our study. The manuscript has been checked carefully to ensure no patient names or identifiable information exists in the text, tables and figures.

4.Consent to participate:

This is a retrospective, non-interventional study that only collects clinical case data of study participants. Since the study participants have been discharged from the hospital and are unable to come to the hospital to sign a written informed consent form, the informed consent for this study will be obtained remotely. Researchers will introduce the study and the content of the informed consent form to participants via phone call or video conference, allow participants sufficient time to understand the form, and patiently answer study-related questions raised by participants. Researchers

will retain audio or video recordings of the informed consent process. Clinical case data of participants can only be collected after obtaining their verbal consent. **All participants in this study have provided informed consent.**

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6.Approval Letter of Ethics : This retrospective study was approved by the Ethics Committee of First Affiliated Hospital of Dalian Medical University for submitting a research paper for publication.The Ethics References No:PJ-KS-KY-2026-682.