Received: 27 January 2022 | Revised: 5 April 2022 | Accepted: 8 April 2022

DOI: 10.1002/jcla.24444

RESEARCH ARTICLE

WILEY

Mutation patterns and prognostic analysis of BRAF/KRAS/PIK3CA in colorectal cancer

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Funding information
We do not have any funding

Abstract

Background and objective: Aberrant gene expression and abnormal signaling pathways often occur in patients with colorectal cancer, in which mutations in B-Raf Proto-Oncogene (BRAF), KRAS Proto-Oncogene (KRAS), and Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha (PIK3CA) are quite common. In this study, the relationship between BRAF, KRAS, and PIK3CA mutations and clinicopathologic features and prognosis of colorectal cancer patients was investigated.

Methods: One hundred and fifty patients with colorectal cancer admitted to Affiliated people's Hospital (Fujian Provincial People's Hospital), Fujian University of Traditional Chinese Medicine were collected and grouped according to the mutation patterns of BRAF, KRAS, and PIK3CA. The association between BRAF, KRAS, and PIK3CA mutations and pathological factors (age, sex, etc.) was analyzed using the Chi-square test. Subsequently, survival analysis was performed to screen the impact factors of overall survival time by Kaplan–Meier (K-M) curve, and Cox regression model was established for the selected factors.

Results: BRAF, KRAS, and PIK3CA mutations were not associated with age, sex, and alcoholism. K–M curve and log-rank test results demonstrated that among the factors included in this study, overall survival rate of colorectal cancer patients was only associated with mutation factors. The prognosis of KRAS+/PIK3CA−/BRAF−mutant and KRAS−/PIK3CA−/BRAF+mutant patients was better than that of KRAS+/PIK3CA+/BRAF−mutant patients.

Conclusion: The mutant patterns of BRAF, KRAS, and PIK3CA were not related to the general and clinicopathological features of patients. The mutant pattern could be used as an independent prognostic factor for colorectal cancer.

KEYWORDS
BRAF mutation, colorectal cancer, KRAS mutation, PIK3CA mutation, prognostic analysis

1 | INTRODUCTION

Colorectal cancer is the fourth most common cancer, which is estimated that approximately 1.9 million people have been diagnosed with colorectal cancer worldwide in 2020, accounting for approximately 10% of all cancer diagnoses.[1] Multiple studies worldwide have shown that colorectal cancer is often caused by aberrant gene expression and dysregulated signaling pathways, such as the activation of multiple signaling pathways of epidermal growth factor receptor (EGFR).[2] EGFR is a transmembrane tyrosine kinase receptor, which triggers two main signaling pathways when binds to ligands: RAS-RAF-phosphatidylinositol 3-kinase (MAPK), which is mainly involved in cell proliferation, and phosphatidylinositol 3-kinase (PI3K)-Phosphatase And Tensin Homolog (PTEN)-AKT, which is involved in cell motility and cell survival. B-Raf Proto-Oncogene (KRAS), KRAS Proto-Oncogene (BRAF), and Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha (PIK3CA) mutations are involved in the activation of these two signaling pathways.[3] KRAS is a molecular switch of intracellular signaling pathways, which plays an imperative role in transferring extracellular growth signals into the nucleus. BRAF, a member of the RAF activating enzyme family, is an important downstream effector of KRAS. BRAF is often activated by somatic mutation and mutated BRAF, and stimulation from either EGFR or KRAS is able to effectively activate downstream signal transduction pathways. PI3K is another downstream effector of EGFR that often interacts with KRAS in regulating cellular functions.[4] PIK3CA gene is involved in encoding the catalytic subunit p110a of PI3K, and its mutations are commonly found in colon cancer, gastric cancer, breast cancer, lung cancer, and ovarian cancer.[5]

It has been reported that KRAS, BRAF, and PIK3CA mutations may be associated with a variety of cancers, with KRAS or PIK3CA mutation frequencies between 0% and 8.3% in the study on gastric cancer.[4] In another study on patients with cholangiocarcinoma, KRAS mutation rates ranged from 15.2% to 50%, and 11 patients with PIK3CA mutations were included in the studied 34 cases.[5] In one study of 194 colorectal cancer tumor samples, 62 samples (31.9%) had mutations only in KRAS codons 12 or 13, 10 samples (5.2%) had mutations in BRAF V600E, and 46 samples (23.7%) had mutations in KRAS codons 61–146, NRAS Proto-Oncogene (NRAS), and PIK3CA.[6] It has also been pointed out that KRAS mutation is found early in tumor progression and occurs in approximately 30%–35% of colorectal cancer cases, while the mutation frequencies of BRAF and PIK3CA are 5%–10% and 10%–20%, respectively.[3]

Gene mutation as one of the biomarkers can be used to predict the outcome of colorectal cancer treatment,[7] and many studies on the prognosis of colorectal cancer patients and KRAS, BRAF, and PIK3CA mutations have been performed over the past decade. Bonetti et al.[8] studied patients with TNM stage I colorectal cancer, of which 50% had mutations in KRAS, NRAS, BRAF, PIK3CA, and the mutations occurred mainly in patients with poor prognosis. Another study found that overall survival of colon cancer patients depended on the mutation status of BRAF\ and signal transduction protein concentration.[9]

In this study, 150 patients with colorectal cancer were collected and divided into 3 groups after DNA sequencing: KRAS+/PIK3CA+/BRAF−, KRAS+/PIK3CA−/BRAF−, and KRAS−/PIK3CA−/BRAF+. The differences in clinicopathological features between patients with different mutation patterns were analyzed by Chi-square test. Subsequently, we explored the independent prognostic factors affecting patient's survival by survival analysis and Cox regression analysis.

2 | MATERIALS AND METHODS

2.1 | Subjects

In this study, 150 patients (73 male patients and 77 female patients, aged 30–85 years) with colorectal cancer who were diagnosed by colonoscopy, biopsy, histology, imaging and other examinations in the Affiliated people's Hospital(Fujian Provincial People's Hospital), Fujian University of Traditional Chinese Medicine were enrolled. None of the patients had lesions of other organs. The clinical information of the patients was collected, including age, sex, drinking history, pathological type, stage, and surgical method (if the surgery was performed). The study protocol was approved by the medical ethics committee of Affiliated people's Hospital (Fujian Provincial People's Hospital), Fujian University of Traditional Chinese Medicine. Because it was a retrospective study and there was no clear patient's information, the patient's informed consent was therefore waived.

2.2 | DNA extraction, library construction and hybrid capture

Genomic DNA from leukocytes was extracted using HighPrep ™ Blood & Tissue DNA Kit (MAGBIO) and used as a control group to exclude genes for reproductive variation. FFPE samples were deparaffinized using xylene and genomic DNA was subsequently extracted with MagMAX ™ FFPE DNA/RNA Ultra Kit (Thermo Fisher Scientific). DNA was then quantified using a Qubit4 fluorometer with a Qubit dsDNA BR assay kit (Thermo Fisher), and the quantified results were assessed by a NanoDrop ™ spectrophotometer (Thermo Fisher).

During library construction, genomic DNA was first split using Covaris M220, followed by end repair, 3' end plus A, and adaptor ligation of the fragments using the KAPA Hyper DNA Library Prep Kit (Roche Diagnostics), followed by fragment length screening using Beckman A63881 purified magnetic beads. Afterward, the DNA library was amplified using PCR technology and its results were purified using Beckman A63881 purified magnetic beads to achieve the purpose of amplifying the library.

The library DNA was partially sequence-blocked by xGen Universal Blocking Oligos (Integrated DNA Technologies) and Human cot-1 DNA (Life Technologies), followed by hybrid capture and washing using Dynabeads M-270 (Life Technologies)

and xGen Lockdown Hybridization and Wash Kit (Integrated DNA Technologies). The capture library was amplified by PCR using KAPA HiFi Hotstart Ready Mix (KAPA Biosystems), purified and quantified using KAPA Library Quantification Kit (KAPA Biosystems), followed by fragment size analysis using Bioanalyzer 2100.

2.3 | Sequencing and bioinformatics analysis

Enriched libraries were sequenced on the HiSeq4000 platform software using the log-end reads. Sequence data were demultiplexed by bcl2fastq software and analyzed using Trimmomatic to remove N bases-related data and low-quality data.

SNPs and indel variants were screened by exploring the function using variant sites of VarScan2 or GATK and setting the limit of the mutation frequency of the allele to 0.5%. Common genetic variants were then removed based on the dbSNP database and the Thousand Genomes Project, and then the detected gene mutations were excluded as germline mutations by comparing with the genomic DNA of the patient's leukocytes.

2.4 | Statistical analysis

Statistical analysis was performed using R software. First, the data of the patients included in the study were preliminarily collated and divided into three subtypes: KRAS+/PIK3CA+/BRAF−, KRAS+/PIK3CA−/BRAF−, and KRAS−/PIK3CA−/BRAF+according to the occurrence of mutations in the three genes of KRAS, BRAF, and PIK3CA. The correlation between mutation subtypes and sex, age and clinical characteristics was compared by Chi-square test. Then, survival analysis was performed: R software "survival" package and "survminer" package were used to establish survival model and draw survival curve for variable factor in each category according to overall survival time. Log-rank test was performed for each model and factors affecting survival time were selected. Finally, Cox regression model was established according to the selected factors.

3 | RESULTS

3.1 | Analysis of clinicopathological features

Our study included 150 patients with colorectal cancer, including 73 men and 77 women, with a median age of 56 years and a ratio of the number of alcoholics to non-alcoholic patients of approximately 7:3. In addition, clinical information such as Eastern Cooperative Oncology Group (ECOG) score, type of cancer, TNM stage, and surgical approach (if surgery was performed) of the patients were also collected. After DNA sequencing, we found that among the 150 colorectal cancer patients, 130 patients were positive for KRAS mutation, 30 patients were positive for PIK3CA mutation, and 20 patients were positive for BRAF mutation. Subsequently, we analyzed the characteristics of gene mutations. Among the three types, the number of patients presenting with KRAS+/PIK3CA−/BRAF−was the most, totally 100, followed by the number of KRAS+/PIK3CA+/BRAF−patients, and the number of patients with KRAS−/PIK3CA−/BRAF+was the lowest, accounting for about 13%. The grouping basis, corresponding frequency, and probability of these factors are reflected in Table 1.

TABLE 1 Clinicopathological features of the patients

Feature	*n*	%
Age		
<56	73	49
≥56	77	51
Sex		
Male	71	47
Female	79	53
Intemperance		
Yes	103	69
No	47	31
ECOG score		
0	44	29
1	55	37
2	51	34
T		
T1	40	27
T2	33	22
T3	44	29
T4	33	22
N		
N0	49	33
N1	46	31
N2	55	37
M		
M0	79	53
M1	71	47
Disease		
Rectum cancer	63	42
Colon cancer	87	58
Surgery type		
Surgery	28	19
Surgery+CRT	29	19
NCRT+surgery	22	15
CRT	71	47
Gene mutation pattern		
KRAS+/PIK3CA+/BRAF-	30	20
KRAS+/PIK3CA-/BRAF-	100	67
KRAS-/PIK3CA-/BRAF+	20	13

TABLE 2 Correlation between gene mutation patterns and clinicopathological features

	KRAS+/PIK3CA+/BRAF-		KRAS+/PIK3CA-/BRAF-		KRAS-/PIK3CA-/BRAF+		*p*-value
	n	%	*n*	%	*n*	%	
Age							
<56	14	9	52	35	7	5	0.37
≥56	16	11	48	32	13	9	
Sex							
Male	19	13	44	29	8	5	0.14
Female	11	7	56	37	12	8	
Intemperance							
Yes	22	15	67	45	14	9	0.80
No	8	5	33	22	6	4	
ECOG score							
0	6	4	28	19	10	7	0.11
1	15	10	34	23	6	4	
2	9	6	38	25	4	3	
T							
T1	7	5	27	18	6	4	0.89
T2	7	5	21	14	5	3	
T3	11	7	27	18	6	4	
T4	5	3	25	17	3	2	
N							
N0	13	9	29	19	7	5	0.24
N1	11	7	31	21	4	3	
N2	6	4	40	27	9	6	
M							
M0	16	11	55	37	8	5	0.47
M1	14	9	45	30	12	8	
Disease							
colon	17	11	37	25	9	6	0.15
rectum	13	9	63	42	11	7	

3.2 | Analysis of the relationship between gene mutations and clinicopathological features of patients

In this section, we analyzed whether there were differences between the clinicopathological features of patients with different mutation patterns (Table 2). The results of Chi-square test showed that there were no significant differences in clinicopathologic features such as age ($p = 0.37$), sex ($p = 0.14$), and alcoholism ($p = 0.80$) between the different groups.

3.3 | Survival analysis

The overall survival analysis of patients (Figure 1) showed that the overall survival time of patients was not statistically different in the stratification of age ($p = 0.99$), sex ($p = 0.34$), alcoholism ($p = 0.6$), ECOG score ($p = 0.67$), cancer type ($p = 0.23$), and surgical approach ($p = 0.93$), and the difference in overall survival time was significant in the stratification of mutation pattern subgroups ($p < 0.1$).

To further analyze the relationship between clinicopathological features and survival of patients, different clinicopathological features such as gene mutation pattern, age, sex, and alcoholism were included as factors in the univariate and multivariate Cox regression models in this study. The results (Table 3) showed that different gene mutation patterns were independent factors for predicting the prognosis of colorectal cancer patients, and the risk of death in colorectal cancer patients was 0.083 (95% CI: 0.039–0.18) and 0.44 (95% CI: 0.20–0.98) times higher in the KRAS+/PIK3CA−/BRAF−and KRAS−/PIK3CA−/BRAF+groups, respectively, compared with the KRAS+/PIK3CA+/BRAF−group, indicating that patients with KRAS+/PIK3CA−/BRAF−and KRAS−/PIK3CA−/BRAF+mutation patterns had a better prognosis.

4 | DISCUSSION

In this study, we performed mutation pattern analysis and prognostic analysis of KRAS, BRAF, and PIK3CA in colorectal cancer patients, and the mutations were divided into: KRAS+/PIK3CA+/BRAF−, KRAS+/

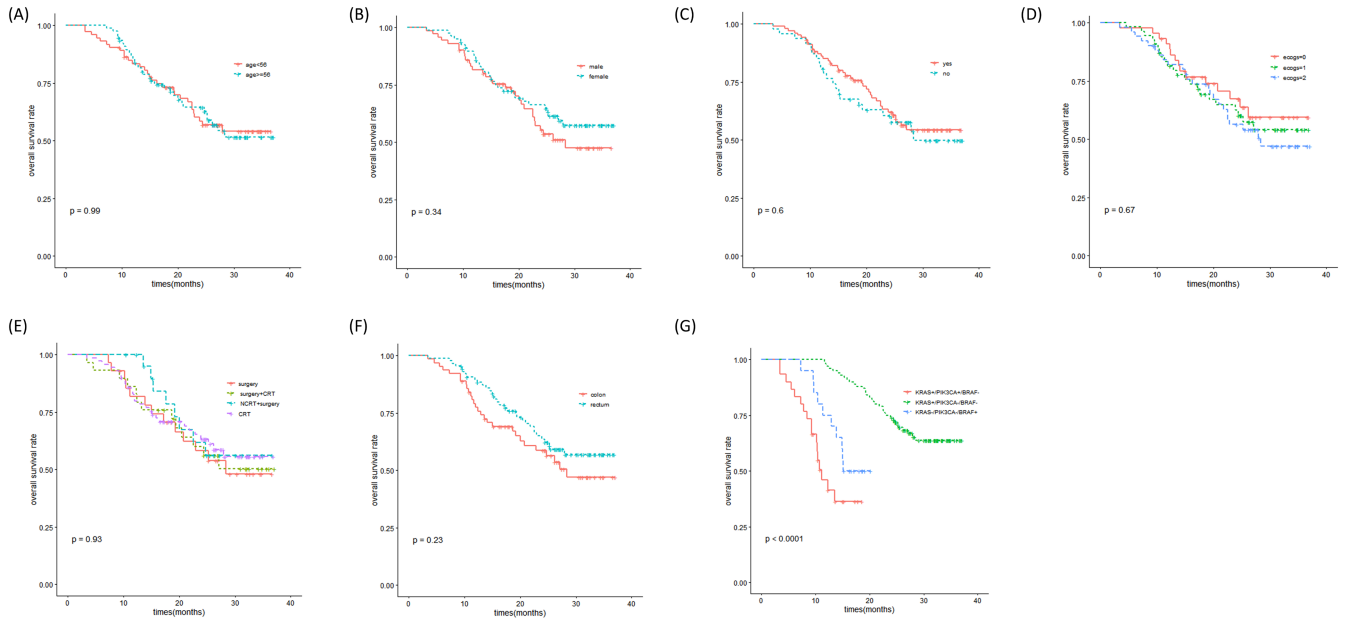

FIGURE 1 Survival analysis of overall survival time of patients with colorectal cancer. A: Survival comparison between patients aged below 56 years and those aged 56 years and above; B: Survival comparison between sex groups; C: Survival comparison between patients with alcoholism or not; D: Survival comparison between ECOG score; E: Survival comparison between surgical methods; F: Survival comparison between rectal cancer and colon cancer; G: survival comparison among three mutation patterns

PIK3CA−/BRAF−, and KRAS−/PIK3CA−/BRAF+. Although the mutation pattern was not remarkably statistically correlated with the clinicopathological features of the patients, it was the only factor in the Cox model in the prognostic analysis.

The RAS-MAPK and PI (3) K signaling pathways are involved in the control of cell proliferation, differentiation, and cell survival, and many studies on colorectal cancer therefore have analyzed mutations in KRAS, BRAF, and PIK3CA.[10] KRAS mutation is the most common, followed by PIK3CA mutation and BRAF mutation in most studies, but prognostic studies on these genes have varied greatly. One study of a prognostic model involving mutations in BRAF, KRAS, PIK3CA, etc., generates the conclusion that patients with mutations in the BRAF gene, exons 12–13 of the KRAS gene, exons 61–146 of the KRAS gene or NRAS or PIK3CA present progressively worse prognoses.[6] Another study was conducted in 229 patients with colorectal cancer to investigate the prognosis of BRAF gene and KRAS gene mutations by survival analysis, and concluded that BRAF gene mutation could be used as a separate prognostic factor.[11] Relevant study also found that PIK3CA mutation status was not the prognostic factor in colorectal cancer patients.[12] Taieb et al.[13] reported that in microsatellite-stable (MSS) stage III colon cancer patients, who represent 90% of the overall stage III population,KRAS and BRAF mutations are prognostic molecular biomarkers of shorter time to recurrence (TTR), shorter survival after relapse (SAR) and overall survival (OS). Li et al.[3] also found that the dual mutations of KRAS and PIK3CA rendered patients with colorectal cancer more vulnerable to liver metastases. Above-mentioned findings suggest that mutations in the KRAS, BRAF, or PIK3CA oncogenes have an important part in the progression of colorectal cancer.

BRAF and KRAS mutations are mutually exclusive. Several reports have discovered a presence of mutations of both BRAF and PIK3CA in 13% of colorectal cancer patients, and a presence of mutations of both BRAF and PTEN in 22% of patients.[14–16] A study by Li et al.[3] on KRAS, BRAF, and PIK3CA mutations in colorectal cancer patients found that some cases with PIK3CA gene mutation were accompanied by KRAS gene mutation, but PIK3CA gene was not simultaneously mutated with BRAF gene. Based on this, this study combined the occurrence of mutations and classified the genotypes into three mutation patterns: KRAS+/PIK3CA+/BRAF−, KRAS+/PIK3CA−/BRAF−, and KRAS−/PIK3CA−/BRAF+, and included them as factors in the study. When studying the relationship between mutation and other factors, this study detected the statistical correlation according to the feature selection Chi-square analysis of data. The test results showed that the mutation type was not correlated with age, sex, ECOG score, and other factors, which were the same as those of YOKOTA et al.[17] One study on colorectal cancer demonstrating that Dukes' staging, histological type, age, and gender have nothing to do with the status of BRAF mutation, the connection between KRAS, PIK3CA mutations, and Dukes' staging does exist.[3] However, in the study by Guo et al., BRAF gene mutation was correlated with TNM stage,[2] but this association could not be reflected in this paper when studying mutation subtypes and stages.

In addition, we also analyzed the prognosis of colorectal cancer patients. First, age, sex, alcohol consumption, ECOG score, T stage, N

TABLE 3 Univariate and multivariate Cox regression results of overall survival time

	Univariate analysis			Multivariate analysis		
	HR	95%CI	*p*	HR	95%CI	*p*
Age			0.99			
<56	1					
≥56	1	(0.6–1.6)				
Sex			0.34			
Male	1					
Female	0.78	(0.47–1.3)				
Intemperance			0.60			
Yes	1					
No	1.2	(0.68–2)				
ECOG score			0.67			
0	1					
1	1.2	(0.62–2.3)				
2	1.3	(0.7–2.6)				
Disease			0.23			
Rectum cancer	1					
Colon cancer	0.74	(0.44–1.2)				
Treatment			0.93			
Surgery	1					
Surgery+CRT	0.95	(0.44–2.1)				
NCRT+surgery	0.76	(0.31–1.8)				
CRT	0.86	(0.44–1.7)				
Mutation pattern			<0.01			<0.01
KRAS+/PIK3CA+/BRAF-	1			1		
KRAS+/PIK3CA-/BRAF-	0.083	(0.039–0.18)		0.083	(0.039–0.18)	
KRAS-/PIK3CA-/BRAF+	0.44	(0.2–0.98)		0.44	(0.2–0.98)	

stage, M stage, cancer types, and mutation subtypes were screened. Kaplan–Meier survival analysis and log-rank test revealed that the influencing factors of overall survival time were mutation subtypes. Therefore, a Cox regression model was established between overall survival time and mutation subtypes. The results of the model indicated that colorectal cancer patients with KRAS+/PIK3CA−/BRAF−and KRAS−/PIK3CA−/BRAF+had a better prognostic effect than patients with KRAS+/PIK3CA+/BRAF−.

In summary, no remarkable statistical correlation was found between KRAS, BRAF, and PIK3CA mutation patterns and clinical features such as sex and age when those mutation patterns were used as factors. The mutation pattern is the only independent prognostic factor affecting overall survival time in colorectal cancer. The novelty of this study is to divide the mutation patterns into subgroups for analysis. More genes can be considered to be included in the mutation pattern for prognostic study to obtain a more accurate prognostic conclusion.

CONFLICT OF INTEREST

We have no disputes of interest.

ETHICAL APPROVAL

Not applicable.

PATIENT CONSENT STATEMENT

Not applicable.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

Not applicable.

CLINICAL TRIAL REGISTRATION

Not applicable.

DATA AVAILABILITY STATEMENT

Please contact corresponding author with reasonable request for the original experimental data and materials.

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How to cite this article: Wang C, Pan D. Mutation patterns and prognostic analysis of BRAF/KRAS/PIK3CA in colorectal cancer. *J Clin Lab Anal*. 2022;36:e24444. doi:10.1002/jcla.24444