

Association between clinicopathological features and survival in patients with primary and paired metastatic colorectal cancer and *KRAS* mutation

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Abstract: The *KRAS* gene mutation is involved in several types of tumors. However, the potential role of the *KRAS* mutation in human primary and paired metastatic colorectal cancer (CRC) among different nationalities is poorly understood. In the present study, we assessed the relationship between *KRAS* mutation status and overall survival (OS) and disease-free survival (DFS) in 230 patients with primary and paired metastatic CRC. The *KRAS* mutation rate in primary CRC tissue was 43.0% (99/230), which was higher than in paired metastatic CRC, which was 31.9% (23/72; $P < 0.001$). Clinicopathologically, the *KRAS* gene mutation rate was higher in tumors that had infiltrated more deeply (T3, T4) and in lymph node (LN) metastases (N1/N2) ($P = 0.029$ and $P = 0.010$, respectively). The *KRAS* gene status did not differ between the Han and Uyghur nationalities in both primary and metastatic CRC. In 72 paired cases, the *KRAS* mutation rate in primary CRC was significantly higher than in metastatic CRC ($P < 0.001$) and in metastatic CRC that had infiltrated more deeply (T3, T4) ($P = 0.034$). In the metastatic cases, the *KRAS* gene mutation rate was higher in patients aged over 65 years ($P = 0.035$). Specifically, *KRAS* mutation was correlated with a poorer OS and DFS ($P = 0.004$ and $P = 0.029$, respectively). In our study, 35 patients with wild-type *KRAS* who received cetuximab targeted therapy had a better DFS than patients with mutant *KRAS* ($P = 0.029$). The results of the current study demonstrate that the *KRAS* status is significantly associated with infiltrating LN metastases and the TNM stage in primary CRC. In addition, the results show that the *KRAS* mutation is significantly more common in primary tumors than in paired metastatic CRC, and the *KRAS* mutation is correlated with a shorter OS and DFS, as patients with wild-type *KRAS* who received cetuximab experienced a longer DFS.

Keywords: CRC, *KRAS*, primary, metastatic, cetuximab, survival

Introduction

Colorectal cancer (CRC) is the most common tumor worldwide, and the World Health Organization (WHO) has declared that it is the third most frequent cancer in men and the second most frequent cancer in women. Epidemiological studies have found that the incidence rates of CRC correlate with geographic location. CRC is a heterogeneous disease evolving from the accumulation of genetic and epigenetic modifications,¹ and *KRAS* homology from the mammalian *ras* gene family is considered a molecular switch that results in aberrant cell growth upon activation.² Specifically, mutations within *KRAS* lead to the constitutive activation of the *EGFR* signaling pathway,³ and the cumulative survival rate of patients with wild-type *KRAS* is significantly higher than that of patients with mutations in this gene. A previous study has showed that the survival rate of patients with the wild-type *KRAS* gene receiving *EGFR* antibody

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therapy was significantly higher than that of patients harboring mutants.⁴ Moreover, a large phase III clinical study has showed that the codons 12 and 13 of exon 2 of the *KRAS* gene correlate with blocked *EGFR* gene monoclonal antibody status with cetuximab and panitumumab and that patients with wild-type *KRAS* benefit the most from *EGFR* antibody therapy.⁵

In recent years, reports on the heterogeneity and ethnic differences between individuals for the *KRAS* gene have been conflicting. Specifically, the *KRAS* gene has been shown to differ between primary cancers and metastases. Moreover, approximately 50% of patients harbor wild-type *KRAS*, but the efficacy of *EGFR* antibody treatment for these patients remains unclear, which may be related to the aforementioned heterogeneity in the *KRAS* gene between the primary tumor and metastatic lesions.^{6–8} In addition, *KRAS* mutations are less common in Asian populations than in black and Caucasian populations, but the *KRAS* gene status has not been delineated by nationality.⁹ The Xinjiang region in the People's Republic of China is located in central Asia and is landlocked. The Uyghur people are the unique minority in Xinjiang, and we sought to identify possible differences in the *KRAS* gene status between Han and Uyghur people in that region. Furthermore, we investigated correlations between the *KRAS* gene status and the clinical characteristics and living conditions of patients with CRC.

Some studies have shown that *KRAS* mutations are associated with a poorer survival in patients with CRC,¹⁰ whereas other studies have reported that *KRAS* mutation does not have a prognostic value or any association with survival in patients with metachronous or synchronous metastatic CRC.^{11,12} We herein analyze the relationship between *KRAS* mutation and survival status in patients from Xinjiang with CRC.

Enrolled patients had histologically proven CRC and had not undergone previous chemotherapy, excluding adjuvant or targeted therapy. The following clinicopathological parameters were recorded: sex, age, ethnicity, differentiation, tumor infiltration, TNM stage, lymph node (LN) involvement, sites of metastasis, tumor location, *KRAS* mutation status in tumor tissue and chemotherapy regimen. All patients gave permission for the use of their serum and tumor tissue.

Materials and methods

Sampling of CRC cases

This study included 230 randomly selected patients with histologically proven CRC: 72 patients with primary CRC who had corresponding paired metastatic tissues, including

62 patients with LN metastases and 10 patients with distant metastases. The patients were chemotherapy-naïve, excluding adjuvant therapy, and were enrolled between March 2012 and July 2014. The study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University, and patients provided informed consent prior to surgery. The following variables were available for analysis: sex, age, ethnicity, differentiation, tumor infiltration, TNM stage, LN involvement, sites of metastasis and tumor location. The TNM classification was defined according to the 2010 WHO criteria. Inclusion criteria for patients with LN metastases were the following: the proportion of tumor should be 20%, and the tumor cells should be above 200. In this study, there were 83 cases who had LN metastases, but only about 72 cases met the requirements.

In this study, disease-free survival (DFS) was defined as the time from the first cetuximab or chemotherapy to death from any other cause. Overall survival (OS) was defined as the time from the first administration of cetuximab or chemotherapy to death from any cause.

Amplification-refractory mutation system (ARMS) analysis

The ARMS experiment was conducted on the European Molecular Genetics Quality Network quality certification platform. DNA was extracted from 5- to 8- μ m-thick paraffin sections containing a representative portion of the tumor tissue (Qiagen DNA Mini Kit, 51304). The concentration of DNA was 20–50 ng/ μ L. Amplifications were performed using a 5-minute initial denaturation at 95°C, followed by 15 cycles of 25 seconds at 95°C, 20 seconds at 64°C, 20 seconds at 72°C, 31 seconds at 93°C, 35 seconds at 60°C and 20 seconds at 72°C. The Ya Kang Bo Gene mutation detection kit was used to analyze the PCR products in conjunction with the MxPro QPCR Software (Version 4.10). Sample channel fluorescein threshold cycle (Ct) values <28 were interpreted as positive (Figure 1).

Statistical analysis

All data were statistically analyzed using the Statistical Package for the Social Sciences, version 17.0 (SPSS17.0). The correlation between clinicopathological features and *KRAS* status was evaluated using a Chi-squared test. The Cox proportional hazards model was used for univariate and multivariate analyses to identify the independent prognostic factors for OS and DFS. OS and DFS were calculated with the Kaplan–Meier method, and differences in survival rates were analyzed with the log-rank test. Logistic

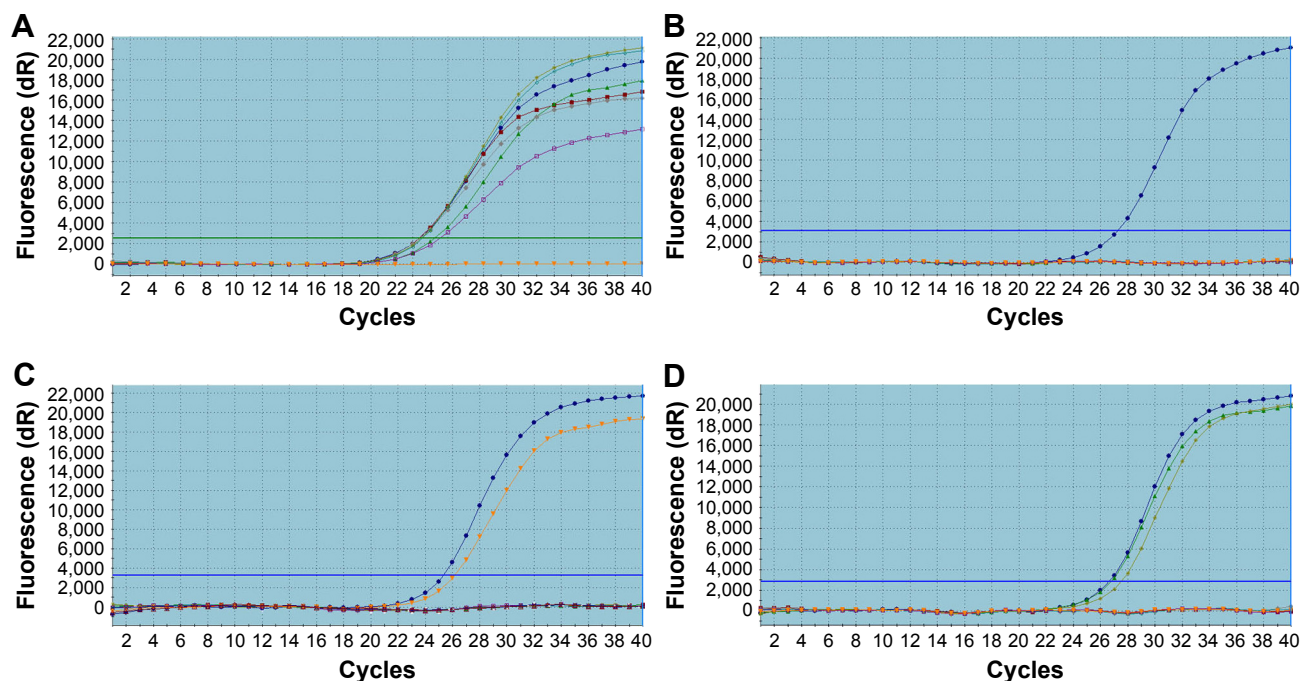


Figure 1 (A) Internal control gene in HEX channel. (B) *KRAS* gene Wild-type in FAM channel. (C) *KRAS* gene mutant-type in FAM channel. (D) *KRAS* gene double mutant-type in FAM channel.

regression models analyzed *KRAS* mutation-site predictors of OS and DFS. A *P*-value of <0.05 was considered to be significant.

Results

KRAS mutations in patients with CRC

In a total of 230 patients with CRC, the *KRAS* mutation rates were 42.6% (75/176) and 44.4% (24/54) for patients of Han and Uyghur descent, respectively. However, this difference was not significant. A total of 99 patients had the *KRAS* gene mutation, corresponding to a total mutation rate of 43.0% (99/230). Specifically, codon 12 was mutated in 84 of these patients (84.9%), whereas codon 13 was mutated in only 15 patients (15.2%). Most primary tumors had single mutations, and only 4 out of 99 tumors (4.0%) harbored a double mutation. The most common mutation site was G12D, which was mutated in 38.4% of tumors (38/99) (Figure 2). In a total of 122 patients with colon cancer, the *KRAS* mutation rates in the left and right sides were 44.0% (33/75) and 59.5% (22/37), respectively. However, the bilateral and transverse *KRAS* gene mutation rates did not significantly differ (Tables 1 and 2).

A comparison of patients having only a primary lesion with those having paired metastatic lesions showed that the *KRAS* gene mutation rate was higher in tumors that had infiltrated more deeply (T3, T4) and in LN metastases

(N1/N2) and metastases (M1) ($P<0.001$) (Table 2). Among a total of 72 paired metastatic tissues, including 62 LN metastases and 10 liver or lung metastases, the *KRAS* mutation rates in the CRC tissues from the Han and Uyghur patients were 34.0% (17/50) and 27.3% (6/22), respectively, but this difference was not significant. All mutations were located at codon 12 (100.0%, 23/23), and the most common mutation site was G12D, which was mutated in 45.5% of all mutations (10/22). The mutation rate was 27.4% (17/62) in LN metastases and 60.0% (6/10) in non-metastatic LNs. Finally, the mutation

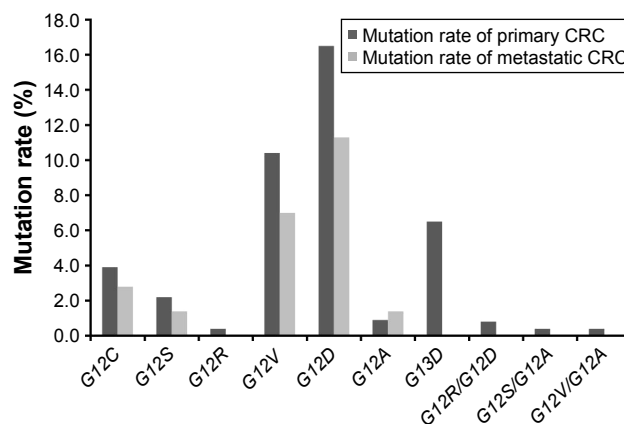


Figure 2 *KRAS* gene single mutation rate of codons 12 and 13 in primary and metastatic CRC.

Abbreviation: CRC, colorectal cancer.

Table 1 KRAS gene mutation location in primary tumors and metastases

KRAS mutation site	N	Primary CRC				N	Metastatic CRC	
		Left	Right	Transverse	Rectum		LN	Liver/lung
G12C	9	3	2	0	4	3	1	2
G12S	5	1	2	0	2	1	0	1
G12R	1	1	0	0	0	0	0	0
G12V	24	9	5	1	9	7	4	3
G12D	38	13	8	1	16	11	7	4
G12A	2	1	0	0	1	1	1	0
G13D	15	7	5	0	3	0	0	0
G12R/G12D	2	0	1	0	1	0	0	0
G12S/G12A	1	0	0	0	1	0	0	0
G12V/G12A	1	1	0	0	0	0	0	0

Notes: The KRAS gene mutation rate was 42.6% (98/230) in 230 primary CRC tumors, and G12D mutations were most common, accounting for 16.5% of all samples (38/230) and 38.8% (38/98) of all mutations. Twenty-three of 72 metastatic CRC samples harbored KRAS mutations, corresponding to a mutation rate of 31.9% (23/72); G12D mutations were most common, accounting for 47.8% (11/23) of all mutations and 15.3% of all samples (11/72).

Abbreviations: CRC, colorectal cancer; LN, lymph node.

Table 2 Analysis of the clinical and pathological characteristics of patients with primary tumors, and comparison of patients having only a primary lesion with those having paired metastatic lesions and association between CRC and codons 12 and 13

Parameters	KRAS status		P-value	LN involvement		P-value	Comparison with codons		P-value
	WT	Mut		Yes	No		12	13	
Sex			0.779			0.800			0.718
Male	73	57		46	84		49	8	
Female	58	42		37	63		35	7	
Age (years)			0.626			0.248			0.929
≥65	90	65		50	105		55	10	
<65	41	34		30	45		29	5	
Ethnic			0.812			0.624			0.457
Han	101	75		62	114		62	13	
Uyghur	30	24		21	33		22	2	
Differentiation			0.626			0.755			0.004
High/moderate	90	65		57	98		60	5	
Poor	41	34		26	49		24	10	
Tumor infiltrating			0.029			<0.001			0.181
T1/T2	48	23		11	60		17	6	
T3/T4	83	76		72	87		67	9	
TNM stage			0.041			<0.001			0.017
I/II	85	51		18	118		39	12	
III/IV	46	48		65	29		45	3	
LN involvement			0.010						0.007
N0	93	54		0	147		41	13	
N1/N2	38	45		83	0		43	2	
Metastasis			0.900			<0.001			0.767
M0	121	91		6	141		78	13	
M1	10	8		77	6		6	2	
Localization			0.143			0.789			0.179
Colon	64	58		45	77		46	12	
Rectum	67	41		38	70		38	3	
Localization (colon)			0.156			0.513			0.516
Left	42	33		25	50		26	7	
Right	15	22		15	22		16	6	
Transverse	7	3		5	5		2	0	

Abbreviations: CRC, colorectal cancer; LN, lymph node; TNM, tumor node metastasis; WT, wide-type; Mut, mutation.

Table 3 The relationship between gene status and the clinical and pathological features of CRC metastases stratified by *KRAS* status

Parameters	<i>KRAS</i> status		P-value
	WT	Mut	
Sex			0.927
Male	25	12	
Female	24	11	
Ethnic			0.573
Han	33	17	
Uyghur	16	6	
Age (years)			0.035
<65	34	10	
≥65	15	13	
Differentiation			0.892
High/well	29	14	
Poor	20	9	
Tumor infiltrating			0.034
T1/T2	11	0	
T3/T4	38	23	
TNM stage			0.512
I/II	8	6	
III/IV	41	17	
LN involvement			0.822
N0	4	3	
N1/N2	45	20	
Metastasis			0.148
M0	47	19	
M1	2	4	
Localization			0.040
LN metastases	45	17	
Liver/lung metastases	4	6	

Abbreviations: CRC, colorectal cancer; WT, wide-type; Mut, mutation; TNM, tumor node metastasis; LN, lymph node.

rates were significantly higher in liver or lung metastases than in LN metastases ($P=0.040$) (Table 3).

Relationship between *KRAS* gene mutation and clinicopathological features

In the primary tumor, the *KRAS* gene mutation rate directly correlated with tumor infiltration (T3, T4) and LN metastasis (N1/N2) (47.8%, 76/159, $P=0.029$ and 54.2%, 45/83, $P=0.010$, respectively) but did not significantly correlate with gender, ethnicity, age, tumor differentiation, histological type and the presence of distant metastases. *KRAS* mutations were more common in individuals older than 65 years and were identified in 58.8% (13/28) ($P=0.035$) of patients with metastatic disease. Other clinical and pathological features did not correlate with *KRAS* status (Table 2).

A comparison of patients having only a primary lesion with those having paired metastatic lesions showed that the *KRAS* mutation rate was higher in tumors that had infiltrated

Table 4 Comparison of *KRAS* gene status between primary CRC tumors and metastases of CRC

Primary	Metastases		P-value
	Mut, n (%)	WT, n (%)	
Mut	23 (100.0)	13 (25.7)	<0.001
WT	0	36 (74.3)	

Notes: Paired chi-squared test examining differences in the *KRAS* gene status between primary and metastatic tumors. Thirty-six primary tumors each harbored mutant and wild-type *KRAS*, whereas 23 and 49 metastases harbored mutant and wild-type *KRAS*, respectively. In 13 primary tumors harboring mutant *KRAS*, the paired metastases harbored wild-type *KRAS*. Significant differences are denoted by $P<0.05$.

Abbreviations: CRC, colorectal cancer; WT, wide-type; Mut, mutation.

more deeply (T3, T4) and in LN metastases (N1/N2) and metastases (M1) ($P<0.001$) (Table 2).

We also analyzed the relationship between codons 12 and 13 of the *KRAS* gene with relevant clinicopathological features and found that mutations in codon 12 were associated with advanced disease; mutations at codon 12 were identified in 84.8% of patients with advanced disease (84/99). Moreover, mutations in codon 13 were associated with poorly differentiated disease; they were identified in 66.7% of poorly differentiated tumors (10/15). Mutations in codon 12 were also associated with late TNM stage, which were identified in 46.4% of samples (39/84), whereas mutations in codon 13 were associated with early TNM stage, and were detected in 80.0% of tissues (12/15). The mutation rate of codon 12 was higher for LN metastases, accounting for 53.6% of all mutations (45/84), but did not include LN metastasis. The mutation rate of codon 13 was higher, accounting for 83.3% (13/15) (Table 3).

Comparison between primary and metastatic CRC

KRAS gene expression was not consistent in the 72 paired primary and metastatic tumors examined in this study. Specifically in the primary tumor, 36 primary tumors expressed mutant *KRAS*, and the other 36 expressed wild-type *KRAS*; in the paired metastases, 23 samples expressed mutant *KRAS*, whereas 49 expressed wild-type *KRAS*. Thirteen primary tumors expressed mutant *KRAS* but the paired metastases expressed wild-type *KRAS*. The mutation rate was significantly higher in the primary tumor than in the metastasis samples ($P<0.001$), and primary tumors and paired metastases showed the same *KRAS* expression (Tables 3 and 4).

KRAS gene status and patient survival analysis

The 30-month survival of the entire patient cohort is shown in Figure 3. Of the total number of patients, 156 (77.8%)

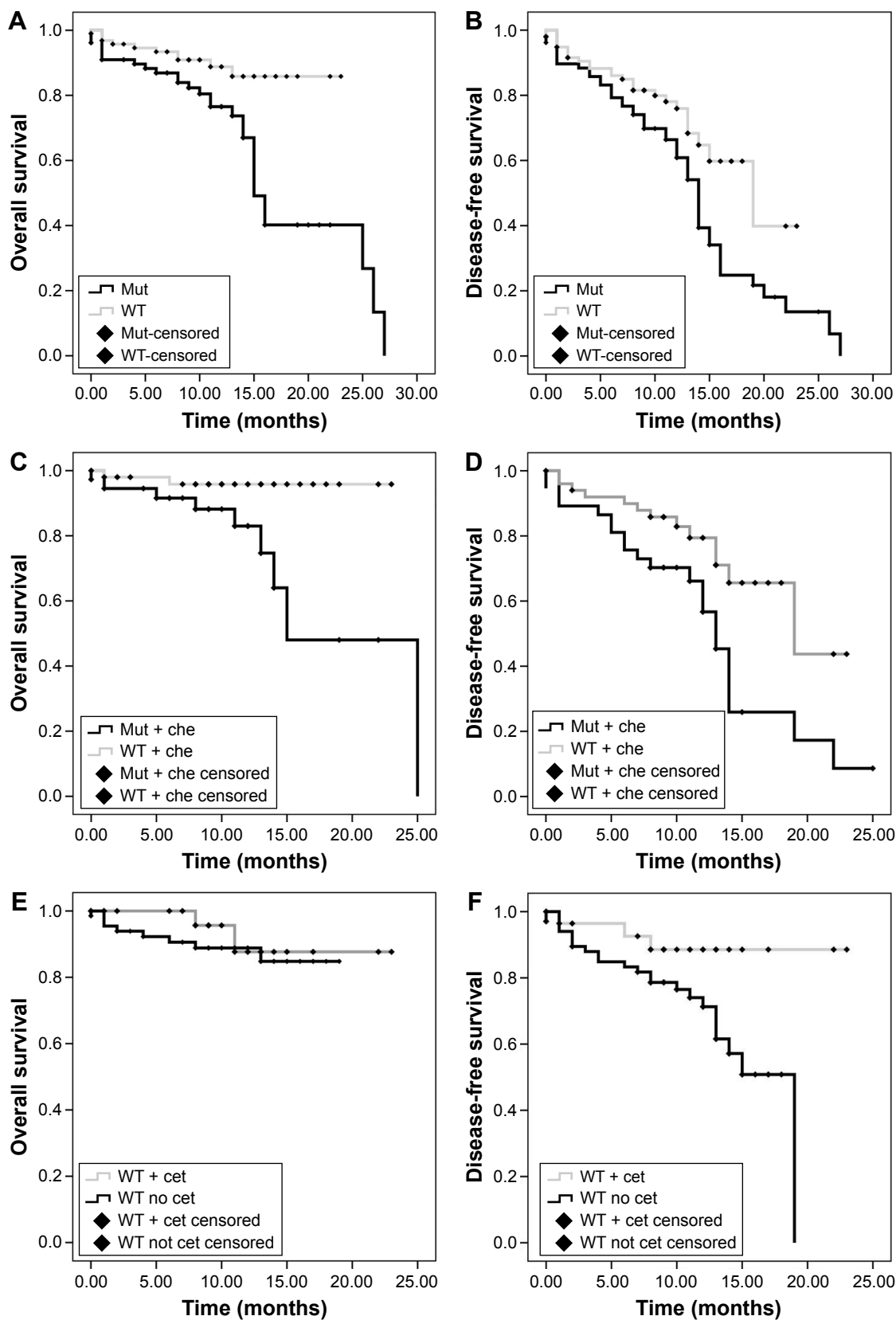


Figure 3 The Kaplan-Meier survival curve for patients with the KRAS gene.

Notes: (A) Overall survival stratified by KRAS Mut and WT. (B) Disease-free survival stratified by KRAS Mut and WT. (C) Overall survival stratified by KRAS status and chemotherapy. (D) Disease-free survival stratified by KRAS status and chemotherapy. (E) Overall survival stratified by KRAS WT and cetuximab. (F) Disease-free survival stratified by KRAS WT and cetuximab.

Abbreviations: che, chemotherapy; cet, cetuximab; Mut, mutation; WT, wild-type.

Table 5 Univariate prognostic analysis of OS and DFS

Parameters	Overall survival		Disease-free survival	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Gender				
Male vs female	1.289 (0.497–3.347)	0.601	1.579 (0.285–1.042)	0.066
Ethnicity				
Han vs Uyghur	0.748 (0.228–2.450)	0.631	1.435 (0.906–2.274)	0.124
Age (years)				
<65 vs ≥65	1.452 (0.557–3.787)	0.446	0.631 (0.339–1.173)	0.146
Differentiation				
PD vs MD + WD	1.024 (0.482–2.2175)	0.951	0.724 (0.417–0.256)	0.251
Tumor infiltration				
T1/T2 vs T3/T4	1.020 (0.353–2.950)	0.971	4.349 (1.531–12.353)	0.006
TNM stage				
I/II vs III/IV	3.039 (0.848–10.896)	0.088	1.637 (0.776–3.455)	0.196
LN involvement				
N0 vs N1/N2	0.308 (0.093–1.026)	0.055	0.808 (0.432–1.509)	0.503
Metastasis				
M0 vs M1	0.856 (0.102–7.200)	0.886	1.241 (0.408–3.777)	0.704
Localization				
Colon vs rectum	0.370 (0.145–0.943)	0.037	0.353 (0.187–0.666)	0.001
KRAS gene status				
Mutant vs wild-type	1.413 (1.181–4.928)	0.004	1.845 (1.125–3.027)	0.010
Codons				
12 vs 13	1.716 (0.506–5.820)	0.386	1.462 (0.612–3.494)	0.373

Abbreviations: OS, overall survival; DFS, disease-free survival; HR, hazard ratio; CI, confidence interval; PD, poor differentiation; MD, moderate differentiation; WD, well differentiated; TNM, tumor node metastasis; LN, lymph node.

were alive and 110 were alive and disease-free, and mutant *KRAS* was associated with a poor OS and DFS ($P=0.004$ and $P=0.010$, respectively). Moreover, 113 patients received chemotherapy, and OS and DFS were longer for patients with wild-type *KRAS* ($P=0.014$ and $P=0.007$, respectively). Among all 35 patients with wild-type *KRAS* and who received cetuximab targeting therapy, 2 patients relapsed and died within 6–10 months, and 5 patients were lost to follow-up. The remaining patients survived, and the targeting therapy improved DFS ($P=0.029$) (Figure 3). Based on the univariate Cox proportional hazards analysis results (Table 5), metastasis and the *KRAS* mutation correlated with a poor OS ($P=0.037$ and $P=0.004$). Furthermore, infiltrating

tumors (T3/T4) and metastasis also correlated with a poor DFS ($P=0.006$ and $P=0.001$, respectively). However, we did not find a worse OS and DFS in patients with LN metastases than those without.

Logistic regression models found that G12V and G12D (odds ratio [OR] =6.049, $P=0.001$ and OR =4.853, $P=0.001$, respectively) were associated with OS, and G12V (OR =4.517, $P=0.003$) with DFS (Table 6).

Discussion

KRAS mutations are important in the carcinogenesis of CRC and play a definitive role in the efficacy of anti-EGFR therapy.^{9,13} In recent years, the association between the

Table 6 Logistic regression models to find out predictors of OS and DFS

Characteristic	OS		DFS	
	OR (95% CI)	P-value	OR (95% CI)	P-value
G12C	1.182 (0.132–10.590)	0.881	0.949 (0.175–5.150)	0.952
G12S	1.181 (0.102–13.612)	0.894	2.807 (0.419–18.823)	0.288
G12R	0	0.999	0	0.999
G12V	6.049 (2.153–16.996)	0.001	4.517 (1.661–4.517)	0.003
G12D	4.853 (1.958–12.027)	0.001	4.530 (1.959–10.479)	<0.001
G12A	4.510 (0.489–41.591)	0.184	4.205 (0.384–46.037)	0.239
G13D	2.364 (0.568–9.839)	0.237	2.373 (0.711–7.917)	0.16

Abbreviations: OS, overall survival; DFS, disease-free survival; OR, odds ratio; CI, confidence interval.

KRAS gene and CRC has been widely studied, and these studies showed that 35%–40% of CRC tumors harbor mutant *KRAS*.^{9,14} The *KRAS* gene mutation occurs in codons 12 and 13, and mutations in codon 12 comprise 80% of all *KRAS* mutations. Our study showed that 43.0% of primary CRC tumors harbored mutants, which corroborated other studies. However, only 32.7% of metastatic tumors harbored mutant *KRAS*, and this incidence is lower than that found in a previous study. *KRAS* gene mutations often occur in codons 12 and 13; a single mutation of G12D is the most common mutation at codon 12. Specifically, Kodaz et al found that the G12D mutation comprised 42.4% of all mutations, with a multiple mutation rate of only 1.1%.¹⁵ Molecular analyses of the primary tumor in patients with metastases have been considered effective in the past because metastases are thought to maintain the biological features of the primary lesions. Specifically, primary and metastatic lesions have been shown to share numerous morphological and immunohistochemical features, allowing pathologists to obtain a diagnosis, and proliferation rates are generally similar in primitive and secondary neoplastic lesions.¹⁶

Yamauchi et al found that rectal cancer, a type of CRC, is more prone to *KRAS* mutations.¹⁷ However, the *KRAS* gene status in our study did not differ between tumors of the colon and rectum, and previous studies examining the *KRAS* gene mutation status in the colon have also reported inconsistencies. For example, Bleeker et al found that the *KRAS* mutation rate is higher in the right side of the colon, whereas Zulhabri et al reported a higher rate in the left side. In this study, the *KRAS* status did not differ between the left, right and transverse colon. In Bleeker et al's study of 55 colon cancer specimens, the *KRAS* gene mutation rates in the left and right colon were 10% (3/29) and 38% (10/26), respectively, whereas in Zulhabri et al's study of 70 colon cancer specimens, these rates were 36.8% (7/19) and 13.7% (7/51), respectively.^{18,19} In our study of 122 colon cancer specimens, the *KRAS* gene mutation rates in the left and right sides were 43.5% (27/62) and 60.0% (18/30), respectively. However, the *KRAS* gene mutation rates did not significantly differ between the bilateral and transverse colon. All previous studies examined a smaller sample, and the patients were from different geographical regions. Conversely, we collected large samples for multivariate analyses to clearly correlate the *KRAS* gene mutation status with the tumor site.

Many studies have examined the relationship between *KRAS* gene status and the clinicopathological features of patients with CRC. Specifically, the *KRAS* gene status has been correlated with patient age: the mutation rate is high

in patients younger than 40 years. However, the *KRAS* gene status of primary CRC tumors did not correlate with age in this study. Nevertheless, *KRAS* mutations were more common in LN metastases, consistent with the findings of Velho et al.²⁰ Tumor infiltration also directly correlated with the *KRAS* mutation rate, but previous studies reported that these parameters did not correlate.²¹ Specifically, previous studies reported that *KRAS* mutation did not correlate with the depth of invasion and LN metastasis in CRC.¹⁵ In our study, patients older than 65 years had a high *KRAS* gene mutation rate, and Kadowaki et al found that survival was low in patients aged older than 65 years.²² Thus, *KRAS* mutation is associated with a poor prognosis. In this study, the correlations between *KRAS* gene mutation status and clinicopathological features were not consistent between primary and metastatic CRC tumors, which may be related to tumor heterogeneity. However, limited amounts of tissue were available for some metastases examined in this study, which may have biased our data.

In our study, the *KRAS* gene mutation rate differed between paired primary tumors and metastases. Specifically, the mutation rates were higher in primary tumors than in metastases, and metastases that harbored *KRAS* mutations were associated with primary tumors that also harbored this mutation, whereas primary tumors harboring this mutation were not necessarily associated with mutant metastases. This finding may be due to heterogeneity within the primary tumor or the acquisition of mutations during the process of metastasis. However, we examined fewer metastatic tumor cells than primary tumor cells, which may have resulted in false negatives. Many current studies have identified *KRAS* heterogeneity between primary tumors and metastases. For example, Siyar Ekinci et al found that the *KRAS* mutation rate is inconsistent between liver or lung metastases and primary tumors; both the primary tumor mutant and paired metastases wild-type also have primary tumor wild-type and paired metastases mutant.^{21,23} Moreover, *KRAS* gene consistency has been reported between primary tumors and metastases, especially non-metastatic LNs.¹⁶ In our study, the *KRAS* gene status was not consistent between primary tumors and LN metastases but identical between primary tumors and liver or lung metastases. Samples of LN metastases are usually small and contain few tumor cells, which may cause false-negative results. However, the LN metastasis specimens in our study were subjected to rigorous screening, and samples containing an insufficient number of tumor cells were excluded. Therefore, the possibility of a false negative is relatively small. Nevertheless, lymphocytes may affect

the expression of *KRAS* gene in the ras signaling pathway,²⁴ which suggests that an autoimmune lymphocyte response may inhibit *KRAS* gene mutation.

Previous studies have found that *KRAS* mutations were associated with a poorer survival. In a Japanese study by Kadowaki et al, *KRAS* and BRAF mutations were associated with a shorter survival,²² whereas another Japanese study revealed that the prognostic impact of *KRAS* mutations on recurrence-free survival was limited in patients with stage II CRC, and *KRAS* mutations were not associated with OS.²⁵ Conversely, our analysis showed that the *KRAS* status affects OS and DFS in patients with CRC: *KRAS* mutations were associated with a shorter OS and DFS compared with wild-type *KRAS*. These results suggest that constitutive *KRAS* mutations may be associated with clinical prognosis in CRC. In our study, cetuximab therapy prolonged DFS but not OS in patients harboring wild-type *KRAS*. However, only 35 patients received the targeted therapy in our study, and 10 patients were lost to follow-up. Therefore, a larger sample is necessary to confirm these findings. Ocvirk et al also found that progression-free survival was significantly longer for patients harboring wild-type *KRAS* tumors than patients harboring mutant *KRAS* tumors.¹⁰

In addition, survival (OS and DFS) did not differ between groups when tumors were stratified by *KRAS* mutation type, that is, mutations in codons 12 and 13, which agrees with findings reported by Huang et al.¹¹ However, the number of samples was limited in our study, and few samples harbored mutations in codon 13. Thus, additional research with larger samples is needed to confirm these findings. In our study, G12V and G12D were associated with a poor prognosis. Bournet et al found that *KRAS* G12D was an independent predictor of a worse prognosis within the entire series and in the subgroup of patients who received chemotherapy for advanced pancreatic adenocarcinoma, but G12V had no obvious effect.²⁶

Conclusion

KRAS mutations were associated with shorter DFS times and more rapid disease progression in patients from Xinjiang, People's Republic of China. Targeted therapy was able to prolong survival for these patients, but this effect was not associated with *KRAS* in Uyghur and Han patients.

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Disclosure

The authors report no conflicts of interest in this work.

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