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Clinicopathologic features and prognostic value of *KRAS*, *NRAS* and *BRAF* mutations and DNA mismatch repair status: A single-center retrospective study of 1,834 Chinese patients with Stage I–IV colorectal cancer

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Mutations of *KRAS*, *NRAS*, *BRAF* and DNA mismatch repair (MMR) status have become an important part of the assessment of patients with colorectal cancer (CRC), while respective clinicopathologic features and prognostic significance in specific stages and related detection strategies remain unclear. We retrospectively analyzed clinicopathologic features and prognosis of 1,834 patients with Stage I–IV colorectal adenocarcinoma. Mutations in *KRAS*, *NRAS* and *BRAF* and DNA MMR status were determined. The mutation rates of *KRAS*, *NRAS* and *BRAF* were 46.4, 3.2 and 3.5%, respectively, and the mismatch repair gene deletion (dMMR) rate was 5.6%. In a multivariate analysis, female, advanced age, tumor type histology, mucinous carcinoma and positive tumor deposits were associated with a high *KRAS* mutation rate. A high *BRAF* mutation rate was associated with female, poor differentiation, lymphovascular invasion and positive tumor deposits. Factors associated with high dMMR rates included low age, large tumor size, poor differentiation, Stages I–III. Tumor site was independently associated with *KRAS* mutation, *BRAF* mutation and dMMR. *KRAS* and *BRAF* mutations were independent risk factors for shorter overall survival (OS) in Stage IV tumors but not in Stage I–III tumors. *NRAS* mutation was an independent risk factor for shorter OS in Stage I–II tumors. dMMR was independently associated with longer OS in Stage III tumors.

Introduction

KRAS, *NRAS* and *BRAF* and DNA mismatch repair (MMR) status have become important biomarkers to evaluate colorectal cancer (CRC). *KRAS* mutations are widely observed in patients with resistance to antiepidermal growth factor receptor (EGFR) therapy and associated with poor prognosis in advanced or recurrent CRC.[1–3] *NRAS* mutations are rare and the clinicopathologic features, prognosis and treatment approaches for patients with *NRAS* mutations are unclear.[4,5] *BRAF* mutations are known as an indicator of poor prognosis and negative predictive biomarkers of anti-EGFR therapy in advanced CRC.[6–10]

Detection strategies and clinical significances of these genes for tumors at specific stages remain unclear since most studies and guidelines focus on patients with recurrence or metastasis and typically detect one or two genes instead of including all the biomarkers above. Accordingly, the prognostic value of mutations at relatively early stages and utility of gene detection as a supplement to the TNM staging system are unclear.

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What's new?

Mutations in KRAS, NRAS, BRAF and DNA mismatch repair (MMR) status are important biomarkers in the assessment of patients with colorectal cancer (CRC). However, the clinicopathologic features associated with these mutations—and their impact on prognosis—are unclear, especially at earlier stages of CRC. In this large Chinese study, the authors analyzed variables such as gender, age, tumor histology, lymphovascular invasion, etc., that were associated with particular oncogene mutations and overall survival. These results should provide guidance for improved clinical strategies and enhance the usefulness of these biomarkers.

We conducted a large retrospective study of cases with *KRAS*, *NRAS*, *BRAF* and MMR data at Fudan University Shanghai Cancer Center over the past 5 years to explore clinicopathologic features and prognosis. The results of our study can provide guidance for development of clinical strategies for gene detection.

Materials and Methods

Patients

A database of patients underwent surgical treatment at the Department of Colorectal Surgery at the Shanghai Cancer Center from January 2013 to June 2018 was retrospectively reviewed. Gene information was found in 2,340 patients and 506 of them were confirmed with incomplete information of gene detection or clinicopathologic features. In total, 1,834 patients were included in the analysis. The treatment plans were designed based on the updated Chinese Ministry of Health guidelines for diagnosis and treatment of CRC and international guidelines.

Our study was conducted according to the principles of the Declaration of Helsinki and was approved by the Research Ethics Committee of the Fudan University Shanghai Cancer Center in China. All patients provided written informed consent for the use of their cancer tissue blocks for molecular analyses.

Mutation screening

The Department of Pathology of Fudan University Shanghai Cancer Center performed mutation detection in all cases using surgical cancer tissues. Sequencing was performed in 1,374 cases. *KRAS* exons 2–4, *NRAS* exons 2–4 and *BRAF* exon 15 were evaluated by bidirectional sequence using ABI 3730XL and a BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA). Three independent experiments were performed to confirm the positive samples. DNA from the other 460 patients was tested using the AmoyDx KRAS/NRAS/BRAF Mutations Detection Kit (Amoy Diagnostics, Xiamen, China) under the principle of the amplification refractory mutation system (ARMS), covering the detection of KRAS mutations (exons 2–4), NRAS mutations (exons 2–4) and BRAF V600 mutations (exon 15). All results were confirmed according to the criterion suggested by the manufacturer.

Immunohistochemistry

Mismatch repair gene deletion (dMMR) was determined by the absence of protein expression for any one of several genes, including hMLH1, hMSH2, hMSH6 or hPMS2. Immunohistochemistry was performed using the fully automated BenchMark ULTRA platform (Ventana Medical Systems, Inc., Tucson, AZ). Normal tissues adjacent to the tumor or lymphocytes in the stroma served as internal positive controls. Each result was confirmed by at least two experienced pathologists.

Table 1. Clinical characteristics of 1,834 patients

Variables	*n* (%)
Sex	
Male	1,088 (59.3)
Female	746 (40.7)
Age	60.2 ± 11.9
Tumor site	
Cecum	43 (2.3)
Ascending colon	277 (15.1)
Hepatic flexure	70 (3.8)
Transverse colon	76 (4.1)
Splenic flexure	40 (2.2)
Descending flexure	71 (3.9)
Sigmoid colon	437 (23.8)
Rectum	805 (43.9)
Multisite tumors	15 (0.8)
Tumor size (cm)	4.3 ± 1.9
TNM stage	
I	192 (10.5)
II	502 (27.4)
III	758 (41.3)
IV	382 (20.8)
Histological	
Ulcer type	1,219 (66.5)
Tumor type	532 (29.0)
Invasive type	83 (4.5)
Pathology	
Adenocarcinoma	1,645 (89.7)
Mucinous carcinoma	189 (10.3)
Differentiation	
G3–G4	557 (30.4)
G1–G2	1,277 (69.6)
Lymphovascular Invasion +	698 (38.1)
Perineural Invasion +	694 (37.8)
Extranodal tumor deposit +	401 (21.9)
KRAS mutant	851 (46.4)
NRAS mutant	58 (3.2)
BRAF mutant	65 (3.5)
dMMR	102 (5.6)

Table 2. Univariate analysis of clinicopathologic features

	KRAS			*NRAS*			*BRAF*			MMR		
	Wild-type	Mutant		Wild-type	Mutant		Wild-type	Mutant		pMMR	dMMR	
Variables	***n* = 983**	***n* = 851**	***p*-value**	***n* = 1,776**	***n* = 58**	***p*-value**	***n* = 1,769**	***n* = 65**	***p*-value**	***n* = 1,732**	***n* = 102**	***p*-value**
Sex			**0.008**			0.70			**0.001**			0.47
Male	611 (56.2)	477 (43.8)		1,055 (97.0)	33 (3.0)		1,062 (97.6)	26 (2.4)		1,024 (94.1)	64 (5.9)	
Female	372 (49.9)	374 (50.1)		721 (96.7)	25 (3.3)		707 (94.8)	39 (5.2)		708 (95.0)	38 (5.0)	
Age	59.5 ± 11.9	60.8 ± 11.9	**0.023**	60.1 ± 12.0	61.8 ± 9.4	0.17	60.1 ± 11.8	60.0 ± 13.9	0.96	60 ± 11.7	55.2 ± 14.1	**0.001**
Tumor site[1]			0.061			0.170			**0.001**			**0.001**
Cecum	15 (34.9)	28 (65.1)		41 (95.3)	2 (4.7)		41 (95.3)	2 (4.7)		39 (90.7)	4 (9.3)	
Ascending colon	115 (41.5)	162 (58.5)		269 (97.1)	8 (2.9)		256 (92.4)	21 (7.6)		242 (87.4)	35 (12.6)	
Hepatic flexure	34 (48.6)	36 (51.4)		69 (98.6)	1 (1.4)		65 (92.9)	5 (7.1)		56 (80.0)	14 (20.0)	
Transverse colon	43 (56.6)	33 (43.4)		75 (98.7)	1 (1.3)		73 (96.1)	3 (3.9)		63 (82.9)	13 (17.1)	
Splenic flexure	22 (55.0)	18 (45.0)		40 (100)	0 (0)		38 (95.0)	2 (5.0)		35 (87.5)	5 (12.5)	
Descending flexure	46 (64.8)	25 (35.2)		70 (98.6)	1 (1.4)		67 (94.4)	4 (5.6)		63 (88.7)	8 (11.3)	
Sigmoid colon	279 (63.8)	158 (36.2)		423 (96.8)	14 (3.2)		427 (97.7)	10 (2.3)		432 (98.9)	5 (1.1)	
Rectum	423 (52.5)	382 (47.5)		774 (96.1)	31 (3.9)		787 (97.8)	18 (2.2)		790 (98.1)	15 (1.9)	
Tumor size (cm)	4.2 ± 1.9	4.4 ± 2.0	**0.026**	4.3 ± 2.0	3.8 ± 1.9	**0.049**	4.3 ± 1.9	4.6 ± 1.9	0.26	4.2 ± 1.9	5.9 ± 3.0	**0.001**
T stage			0.56			0.76			0.80			0.82
T1	32 (57.1)	24 (42.9)		53 (94.7)	3 (5.3)		54 (96.5)	2 (3.5)		54 (96.5)	2 (3.5)	
T2	135 (57.2)	101 (42.8)		230 (97.5)	6 (2.5)		228 (96.6)	8 (3.4)		223 (94.5)	13 (5.5)	
T3	550 (52.5)	498 (47.5)		1,015 (96.9)	33 (3.1)		1,014 (96.7)	34 (3.3)		986 (94.1)	62 (5.9)	
T4	266 (53.8)	228 (46.2)		478 (96.7)	16 (3.3)		473 (95.7)	21 (4.3)		469 (94.9)	25 (5.1)	
N stage			0.15			0.18			**0.005**			**0.001**
N0	421 (55.6)	336 (44.4)		738 (97.5)	19 (2.5)		741 (97.9)	16 (2.1)		693 (91.6)	64 (8.4)	
N+	562 (52.2)	515 (47.8)		1,038 (96.4)	39 (3.6)		1,028 (95.4)	49 (4.6)		1,039 (96.5)	38 (3.5)	
M stage			0.22			0.76			**0.044**			**0.002**
M0	789 (54.3)	663 (45.7)		1,407 (96.9)	45 (3.1)		1,407 (96.8)	45 (3.2)		1,359 (93.6)	93 (6.4)	
M1	194 (50.8)	188 (49.2)		369 (96.7)	13 (3.3)		362 (94.9)	20 (5.1)		373 (97.5)	9 (2.5)	
TNM stage			0.27			0.35			**0.014**			**0.001**
I	115 (59.9)	77 (40.1)		184 (95.9)	8 (4.1)		184 (95.9)	8 (4.1)		181 (94.3)	11 (5.7)	
II	270 (53.7)	232 (46.3)		492 (98.0)	10 (2.0		495 (98.6)	7 (1.4)		452 (90.1)	50 (9.9)	
III	404 (53.3)	354 (46.7)		731 (96.4)	27 (3.6)		728 (96.0)	30 (4.0)		726 (95.8)	32 (4.2)	
IV	194 (50.8)	188 (49.2)		369 (96.7)	13 (3.3)		362 (94.9)	20 (5.1)		373 (97.5)	9 (2.5)	
Histological	983	851	**0.001**			0.21			0.52			0.15
Ulcer type	691 (56.7)	528 (43.3)		1,177 (96.5)	42 (3.5)		1,176 (96.5)	43 (3.5)		1,155 (94.7)	64 (5.3)	
Tumor type	244 (45.9)	288 (54.1)		518 (97.4)	14 (2.6)		514 (96.6)	18 (3.4)		496 (93.2)	36 (6.8)	
Invasive type	48 (57.8)	35 (42.5)		81 (97.6)	2 (2.4)		79 (95.2)	4 (4.8)		81 (97.6)	2 (2.4)	

(Continues)

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Table 2. Univariate analysis of clinicopathologic features (Continued)

	KRAS			***NRAS***			***BRAF***			**MMR**		
	Wild-type	**Mutant**		**Wild-type**	**Mutant**		**Wild-type**	**Mutant**		**pMMR**	**dMMR**	
Variables	***n* = 983**	***n* = 851**	***p*-value**	***n* = 1,776**	***n* = 58**	***p*-value**	***n* = 1,769**	***n* = 65**	***p*-value**	***n* = 1,732**	***n* = 102**	***p*-value**
Pathology			**0.001**			0.68			0.17			**0.001**
Adenocarcinoma	898 (54.6)	747 (45.4)		1,592 (97.1)	53 (2.9)		1,590 (96.7)	55 (3.3)		1,569 (95.4)	76 (4.6)	
Mucinous carcinoma	85 (45.2)	104 (54.8)		184 (97.4)	5 (2.6)		179 (94.7)	10 (5.3)		163 (86.2)	26 (13.8)	
Differentiation			0.17			0.70			**0.001**			**0.001**
G3–G4	291 (52.2)	266 (47.8)		541 (97.1)	16 (2.9)		518 (93.0)	39 (7.0)		502 (90.1)	55 (9.9)	
G1–G2	692 (54.2)	585 (45.8)		1,235 (96.7)	42 (3.3)		1,251 (98.0)	26 (2.0)		1,230 (96.3)	47 (3.7)	
Lymphovascular Invasion			0.77			0.58			**0.001**			0.17
Negative	615 (54.1)	521 (45.9)		1,101 (97.0)	35 (3.0)		1,108 (97.5)	28 (2.5)		1,064 (93.7)	72 (6.3)	
Positive	368 (52.7)	330 (47.3)		675 (96.7)	23 (3.3)		661 (94.7)	37 (5.3)		668 (95.7)	30 (4.3)	
Perineural Invasion			0.41			0.40			0.07			**0.002**
Negative	618 (54.2)	522 (45.8)		1,107 (97.1)	33 (2.9)		1,106 (97.0)	34 (3.0)		1,062 (93.2)	78 (6.8)	
Positive	365 (52.6)	329 (47.4)		669 (96.3)	25 (3.7)		663 (95.5)	31 (4.5)		670 (96.5)	24 (3.5)	
Extranodal tumor deposit			0.055			0.52			**0.001**			**0.002**
Negative	785 (54.8)	648 (45.2)		1,390 (97.0)	43 (3.0)		1,394 (97.3)	39 (2.7)		1,341 (93.6)	92 (6.4)	
Positive	198 (49.3)	203 (50.7)		386 (96.3)	15 (3.7)		375 (93.5)	26 (6.5)		391 (97.5)	10 (2.5)	

[1]Another 15 patients with multisite tumors were excluded.

Table 3. Multivariate analysis of clinicopathologic features

	KRAS mutant			*BRAF* mutant			dMMR		
Variables	**OR**	**95%CI**	***p*-value**	**OR**	**95%CI**	***p*-value**	**OR**	**95%CI**	***p*-value**
Sex									
Female	1	Ref		1	Ref				
Male	0.81	0.66–0.99	**0.045**	0.57	0.34–0.97	**0.039**			
Age	1.01	1.01–1.02	**0.005**				0.97	0.95–0.98	**0.001**
Tumor site	0.92	0.88–0.96	**0.001**	0.81	0.73–0.90	**0.001**	0.71	0.64–0.78	**0.001**
Tumor size							1.29	1.17–1.42	**0.001**
Histology									
Ulcer type	1	Ref							
Tumor type	1.63	1.31–2.04	**0.001**						
Invasive type	0.91	0.53–1.56	0.726						
Pathology									
Adenocarcinoma	0.66	0.47–0.94	**0.021**						
Mucinous carcinoma	1	Ref							
Differentiation									
G3–G4				2.33	1.31–4.14	**0.004**	2.74	1.66–4.51	**0.001**
G1–G2				1	Ref		1	Ref	
Lymphovascular Invasion				1.86	1.02–3.50	**0.043**			
Perineural Invasion									
Extranodal tumor deposit	1.39	1.10–1.76	**0.008**	2.28	1.29–4.05	**0.005**			
TNM Stage									
I							13.71	4.64–40.45	**0.001**
II							8.55	3.58–20.47	**0.001**
III							2.92	1.22–6.98	**0.016**
IV							1	Ref	

Statistical analysis

All statistical analyses were performed by SPSS version 25.0 (IBM Corporation, Armonk, NY). Chi-squared tests or Fisher's exact tests for categorical variables were used to compare the mutation status and clinical features. The Kolmogorov–Smirnov test was used to verify the normal distribution assumptions. The exploratory comparison of normally distributed and nonnormally distributed independent groups was performed using *t*-tests and Mann–Whitney U tests (two groups). Overall survival (OS) was defined as the period of time between the first surgery and death from any cause. Analyses identifying prognostic predictors are performed using Cox proportional hazard models. Ten to fifteen predictors are necessary to proceed with multivariate survival analysis, whereby the selection for independent factors in the multivariate model was based on the univariate results. Log-rank tests were employed to identify the associations between OS and predictors and all results are visualized by survival curves using the Kaplan–Meier method. A two-sided p-value <0.05 was considered statistically significant.

Data availability statement

The data that support the findings of our study are available from the corresponding author upon reasonable request.

Results

Patients and mutations

Basic information for 1,834 patients is summarized in Table 1. One case of both *KRAS* and *NRAS* mutations, two cases of *KRAS* and *BRAF* mutations and three cases of *NRAS* and *BRAF* mutations were excluded from the prognostic analysis.

Clinicopathologic features

Univariate analyses of clinicopathologic features according to mutations in *KRAS*, *NRAS* and *BRAF* and DNA MMR status are listed in Table 2.

Results of the multivariate analysis are summarized in Table 3. Only tumor size was associated with *NRAS* mutations in the univariate analysis. Therefore, *NRAS* mutations were excluded from the multivariate analysis. *KRAS* mutation rate was high for the following factors: female, advanced age, tumor type histology, mucinous carcinoma and positive tumor deposits. *BRAF* showed a high mutation rate in female, poor differentiation, lymphovascular invasion and positive tumor deposits. A high rate of dMMR was associated with low age, large tumor size, poor differentiation and Stages I–III. Tumor site was independently associated with *KRAS* mutation, *BRAF* mutation and dMMR.

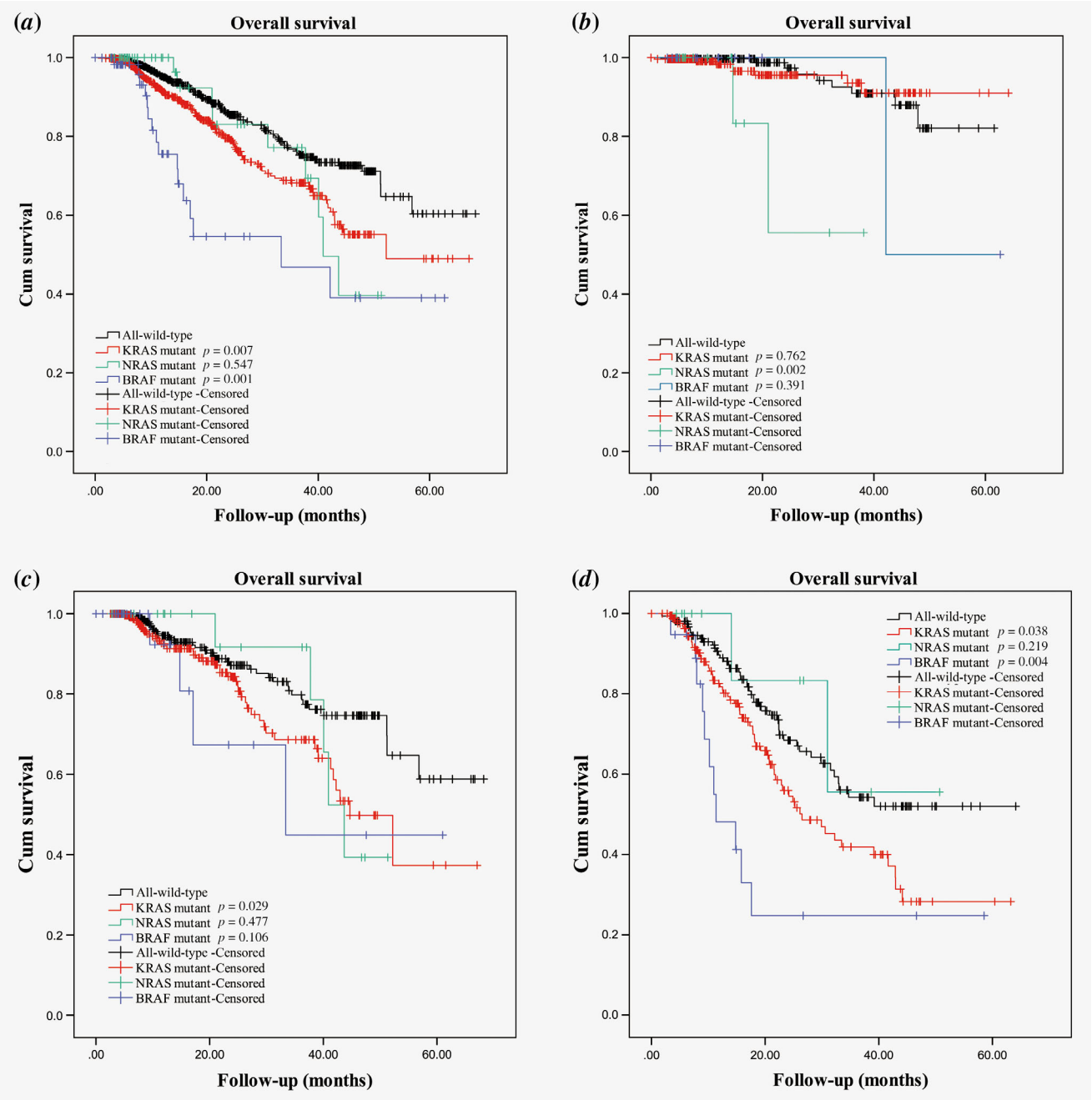

Figure 1. Kaplan–Meier analysis of OS at (*a*) Stages I–IV, (*b*) Stages I–II, (*c*) Stage III and (*d*) Stage IV. Each *p*-value reflects its respective mutation compared with all-wild-type.

Survival analysis

In a univariate analysis of Stage I–IV tumors, *KRAS*-mutated tumors and *BRAF*-mutated tumors were associated with a shorter OS compared to that of all-wild-type tumors. There was no significant difference in OS between *NRAS*-mutated tumors and all-wild-type tumors (Fig. 1). Table 4 presents the results obtained from the Cox analysis of prognostic actors.

No differences in OS between *KRAS*-mutated tumors and all-wild-type tumors of Stages I–II were detected in both the univariate analysis and multivariate analysis (Fig. 1 and Table 4). In Stage III, *KRAS*-mutated tumors were associated with a shorter OS than that of all-wild-type tumors in a univariate analysis but not in a multivariate analysis. In Stage IV, we observed a significant difference in OS between *KRAS*-mutated tumors and all-wild-type tumors in both a univariate analysis and multivariate analysis.

In Stages I–II, OS was shorter for *NRAS*-mutated tumors than for all-wild-type tumors in both the univariate analysis and

Table 4. Cox analysis of prognostic actors for OS in patients from Stage I to Stage IV

	Stages I–II		Stage III		Stage IV	
Prognosis variables	***p*-value**	**HR (95% CI)**	***p*-value**	**HR (95% CI)**	***p*-value**	**HR (95% CI)**
Sex (female)	**0.088**	0.26 (0.06–1.22)	0.20	0.74 (0.46–1.18)	0.43	1.18 (0.79–1.76)
Age	0.41	1.02 (0.97–1.07)	0.86	1.00 (0.98–1.02)	0.96	1.00 (0.98–1.02)
Tumor site	0.79	0.97 (0.77–1.21)	0.71	1.02 (0.92–1.13)	**0.013**	0.91 (0.84–0.98)
Tumor size	**0.001**	1.44 (1.21–1.73)	**0.001**	1.40 (1.24–1.58)	**0.047**	1.10 (1.01–1.21)
Histology						
Tumor type	0.98	1.02 (0.33–3.12)	0.35	1.28 (0.76–2.15)	0.94	0.94 (0.58–1.53)
Invasive	–	–	0.90	0.94 (0.37–2.42)	0.32	1.44 (0.70–2.99)
Pathology (Mucinous)	0.83	0.75 (0.058–9.64)	0.35	1.33 (0.74–2.42)	0.48	0.79 (0.42–1.50)
Differentiation (G1–G2)	0.98	1.02 (0.26–4.00)	**0.001**	0.30 (0.19–0.49)	**0.007**	0.59 (0.40–0.86)
Lymphovascular Invasion	**0.04**	3.80 (1.06–13.54)	0.18	1.43 (0.85–2.39)	0.29	0.79 (0.51–1.22)
Perineural Invasion	**0.001**	5.56 (2.01–15.41)	**0.008**	1.88 (1.18–2.99)	**0.017**	1.65 (1.09–2.49)
Extranodal tumor deposit	–	–	**0.006**	1.89 (1.20–2.97)	0.14	1.36 (0.91–2.04)
KRAS mutant	0.76	1.20 (0.37–3.91)	0.13	1.47 (0.89–2.42)	**0.022**	1.60 (1.07–2.40)
NRAS mutant	**0.025**	6.13 (1.25–30.01)	0.071	2.29 (0.93–5.66)	0.25	0.42 (0.10–1.81)
BRAF mutant	–	–	0.29	1.78 (0.61–5.22)	**0.003**	2.84 (1.43–5.67)
dMMR	0.20	2.73 (0.60–12.47)	**0.008**	0.12 (0.25–0.58)	0.67	0.72 (0.16–3.26)

Bold values indicate *p*-values less than 0.05.

multivariate analysis. No similar difference was observed in Stage III tumors in the univariate analysis or multivariate analysis or in Stage IV tumors in the univariate analysis or multivariate analysis.

No statistically significant difference in OS between patients with *BRAF*-mutated tumors and all-wild-type tumors was observed for Stages I–II and Stage III in the univariate analysis. For Stage IV, the OS of patients with *BRAF*-mutated tumors was shorter than that of patients with all-wild-type tumors in both univariate analysis and multivariate analysis.

In the Cox analysis, dMMR was independently associated with longer OS in Stage III but not Stage I–II or Stage IV tumors.

Discussion

We retrospectively analyzed mutations in *KRAS*, *NRAS* and *BRAF* and DNA MMR status of 1,834 patients with colorectal adenocarcinoma during the past 5 years at our institution. Comprehensive information, including clinicopathologic features and prognosis, was gathered to explore the necessity and optimization of gene detection for tumors with different clinicopathologic features and stages.

Relevant studies of relatively large populations are summarized in Table 5.[5,7,8,11–22] The *KRAS* mutation rate in our study was similar to those in studies of Eastern and Western populations. As for *NRAS*, a low mutation rate was reported in most studies and only Stage IV cases are included. Interestingly, the *BRAF* mutation rate was lower in our study and other studies of Asian populations than in studies of Western populations. Turning now to DNA MMR status, immunohistochemical analyses and DNA sequencing are not 100% accurate for identifying dMMR/MSI. However, these two methods are highly consistent in results, that is, near 95%.[23] It is difficult to definitively determine the difference in dMMR rate between Eastern and Western populations based on available studies.

Clinicopathologic factors related to a high *KRAS* mutation rate in our study were female, advanced age, tumor type histology, mucinous carcinoma and positive tumor deposits. However, Imamura *et al.* reported different results in a study of 1,267 patients. In their study, *KRAS* mutations were associated with male, well-moderate differentiation, absent-minimal peritumoral lymphocytic reaction.[16] Further studies are expected to explorer clinicopathologic features associated with *KRAS* mutations. No significant differences in clinicopathologic features were observed between *NRAS*-mutated tumors and all-wild-type tumors in our study or other studies. A high *BRAF* mutation rate was associated with female, poor differentiation, lymphovascular invasion and positive tumor deposits in our study. Similar results were found in previous studies.[6,20] This is the first study to report that positive tumor deposits are independently related to *BRAF* mutations. Positive tumor deposits are associated with poor prognosis and have become a reference factor for TNM staging.[24,25] Our findings suggest that a high *BRAF* mutation rate is the main predictor of a poor prognosis in patients with positive tumor deposits. dMMR was relatively common in large, poor differentiated and Stage I–II tumors, as reported in previous studies.[11,22] However, we observed that the age of high incidence of dMMR was different in different population studies. Our results are consistent with other studies of Chinese populations, with a high dMMR rate in young individuals.[26,27] In studies of western populations, high dMMR rates appear to be associated with older age.[28–30]

In general, *KRAS* mutation rate decreased from right colon to left colon, but increased slightly in rectum. *BRAF* mutation rate was higher in right colon than left colon and lowest in rectum.

Table 5. Clinical studies reporting rates of *KRAS*, *NRAS*, and *BRAF* mutations and dMMR/MSI

Year	Author	Journal	Number of centers	Area	Number of patients	Stage	*KRAS* mutant rate	*NRAS* mutant rate	*BRAF* mutant rate	dMMR/MSI rate
2005	Benatti *et al.*	Clin Cancer Res	3	Italy	1,263	I–IV	–	–	–	20.3%
2007	Koopman *et al.*	Br J Cancer	Many (74)	The Netherlands	515	IV	–	–	–	3.5%
2009	Des Guetz *et al.*	Eur J Cancer	Many	Many	3,690	II–III	–	–	–	13.7%
2010	De Roock *et al.*	Lancet Oncol	11	Europe	773	IV	40.0%	2.6%	4.7%	–
2011	Sinicrope *et al.*	J Natl Cancer Inst	Many	Europe and America	2,141	II–III	–	–	–	16.1%
2012	Imamura *et al.*	Clin Cancer Res	Many	U.S	1,261	I–IV	35.3%	–	14.4%	–
2013	Douillard *et al.*	N Engl J Med	Many	Europe and America	1,096	IV	45.6%	7.5%	8.3%	–
2014	Imamura *et al.*	Mol Cancer	Many	U.S	1,267	I–IV	40.0%		14.5%	–
2014	Schirripa *et al.*	Int J Cancer	1	Italy	786	IV	50.0%	6.0%	9.2%	–
2016	Summers *et al.*	Clin Cancer Res	Many	UK and Ireland	2,157	IV	39.8%	4.0%	9.5%	4.6%
2011	Yokota *et al.*	Br J Cancer	1	Japan	229	IV	34.5%	-	6.5%	–
2014	Tong *et al.*	Cancer Biol Ther	1	HK, China	1,506	–	44.5%	–	–	–
2015	Zhang *et al.*	Sci Rep	3	China	1,110	I-IV	45.4%	3.9%	3.1%	–
2015	Kawazoe *et al.*	BMC Cancer	1	Japan	264	IV	37.9%	4.2%	5.4%	–
2015	Yan *et al.*	World J Gastroenterol	1	China	538	I–IV	37.9%	–	4.4%	11.4%
Current study	Guo *et al.*	Int J Cancer	1	China	1,854	I–IV	46.4%	3.2%	3.5%	5.6%

The rate of dMMR increased gradually from cecum to hepatic flexure and then decreased from hepatic flexure to rectum. Yamauchi *et al.* reported that the rate of *KRAS* mutations was highest in cecal tumors and gradually decrease from cecal to transverse colon, but no obvious pattern of *KRAS* mutation rate was found from splenic flexure to rectum in their study.[31] Rosty *et al.* and Imamura *et al.* confirmed the highest *KRAS* mutation rate in cecal tumors.[16,32] Different site distribution of *KRAS* mutations might be caused by the fact that only *KRAS* exon 2 (codons 12 and 13) was sequenced in these previous studies. Yamauchi *et al.* also reported that the rates of MSI-high and *BRAF* mutations gradually increased from the rectum to ascending colon, followed by falls in the cecum, which was similar to the trends in our results.[31]

Considering that mutations in *KRAS*, *NRAS* and *BRAF* and DNA MMR statuses are not all routinely tested in many clinical institutions, we suggest a gene detection strategy to be developed in future studies based on the clinicopathologic differences described above.

Poor prognosis and resistance to anti-EGFR targeted therapy of *KRAS* mutations are defined in Stage IV patients.[33,34] However, in Stage I–III patients, the prognostic value of *KRAS* is controversial. Ogino *et al.* reported that the KRAS mutational status is not associated with DFS or OS in a study of 508 patients with Stage III CRC.[35] Similarly, Roth *et al.* reported that *KRAS* mutations do not have major prognostic value based on a study of 1,404 patients with Stage II–III CRC.[36] However, Hutchins *et al.* found that the risk of recurrence is significantly higher for *KRAS* mutants than wild-type *KRAS* in the QUASAR study, which included 1,708 Stage II cases and 163 Stage III cases.[37] Taieb *et al.* evaluated 4,411 patients with Stage III colorectal and found that *BRAF* or *KRAS* mutations were independently associated with a shorter time to recurrence, survival after recurrence and OS in patients with MSS, but not in MSI tumors.[38] In the univariate analysis, prognosis was worse in *KRAS* mutants than all-wild-type cases and this could be explained by the increase in the rate of *KRAS* mutations as the tumor stage increased in our study (Table 2).

Poor prognosis for *BRAF* mutation has been widely reported in Stage IV cases.[10] Similar results can be found in a few studies of Stage II or III patients.[38,39] Our statistical analyses showed that *BRAF* mutation is an independent risk factor for shorter OS only in Stage IV tumors but not Stage I–III tumors. The contradiction might be explained by the low incidence of *BRAF* mutation in the Asian population. Therefore, far fewer patients with Stage II–III *BRAF*-mutated cancer were included in our study than in previous studies, resulting in statistically insignificant results.

Unlike *KRAS* and *BRAF* mutations, *NRAS* mutation is an independent risk factor for shorter OS in Stages I–II but not in Stage III or IV. A few studies have reported a poor prognosis associated with *NRAS* mutations.[8,40,41] However, very few studies reported the prognostic value of *NRAS* mutations at specific stages. In a retrospective study of patients with Stage IV CRC, Schirripa *et al.* reported that *NRAS* mutations are associated with a shorter OS than all-wild-type cases and more patients with *NRAS* mutations at Stage IV were included in their study than in ours (47 *vs.* 13).[5] Further studies of *NRAS* mutations are needed.

dMMR was an independent prognostic factor for a favorable prognosis for patients with Stage III cancer in our study. Similar conclusions are reached in a number of studies under certain conditions. Sinicrope *et al.* reported that dMMR is significantly associated with better survival after recurrence in patients with Stage III proximal colon cancers.[42] In a study of 1,254 patients with Stage II–IV cancer, Klingbiel *et al.*[43] reported that MSI-H is associated with both longer relapse-free survival and OS in Stage II patients and relapse-free survival in Stage III patients. Differences in conclusions could be explained by different sample size and multivariate analysis methods.

In conclusion, mutations in *KRAS*, *NRAS* and *BRAF* and dMMR were associated with different clinicopathologic features. *KRAS* and *BRAF* mutations were independent risk factors for shorter OS in Stage IV tumors. *NRAS* mutations were an independent risk factor for shorter OS in Stage I–II tumors. dMMR was an independent protective factor for longer OS in Stage III tumors. The clinicopathologic features and prognostic values of these markers require further validation, especially in early-stage patients.

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