Japanese patients with curatively resected CRC.

Key words: Colorectal cancer; *KRAS*; *BRAF*; Microsatellite instability; Prognostic factor

Core tip: Although *KRAS* and *BRAF* mutations play a critical role in colorectal cancer development, little is known regarding the prognostic role of these genetic alterations after adjustment for microsatellite instability status in Asian populations. To the authors' knowledge, the current study is the first large-scale study to clarify the impact of *KRAS* and *BRAF* mutations on the survival outcomes of colorectal cancer in Asian populations. We found that *KRAS* and *BRAF* mutations were separately associated with inferior disease-free survival and overall survival, independent of microsatellite instability status, in patients with curatively resected colorectal cancer.

Kadowaki S, Kakuta M, Takahashi S, Takahashi A, Arai Y, Nishimura Y, Yatsuoka T, Ooki A, Yamaguchi K, Matsuo K, Muro K, Akagi K. Prognostic value of *KRAS* and *BRAF* mutations in curatively resected colorectal cancer. *World J Gastroenterol* 2015; 21(4): 1275-1283 Available from: URL: http://www.wjgnet.com/1007-9327/full/v21/i4/1275.htm DOI: http://dx.doi.org/10.3748/wjg.v21.i4.1275

INTRODUCTION

Colorectal cancer (CRC) develops through diverse mechanisms such as chromosomal instability (CIN), microsatellite instability (MSI), and epigenetic DNA promoter methylation [CpG island methylator phenotype (CIMP)][1]. CIMP and MSI-high (MSI-H) phenotypes are closely associated. Most sporadic MSI-H tumors develop through CIMP-associated methylation of *MLH1*, and *BRAF* mutations occur frequently in both phenotypes[2,3]. *KRAS* mutations mainly occur in CIN and are partly associated with intermediate CIMP epigenotype[4]. *KRAS* and *BRAF* mutations are mutually exclusive; both cause RAS/RAF/MAPK signaling pathway upregulation and are crucial in CRC development.

KRAS encodes a guanosine triphosphate/guanosine diphosphate binding protein; *KRAS* mutations are observed in approximately 30%-40% CRCs[5-8]. *KRAS* mutations are well known as predictive markers of resistance to epidermal growth factor receptor-targeted antibodies in metastatic CRC, but their prognostic value remains controversial. Some studies have shown that *KRAS* mutations are associated with poorer survival in CRC[8,9], while others found no association[6,7].

BRAF encodes a serine/threonine protein kinase, a downstream effector of the KRAS protein. Activating *BRAF* mutations occur in approximately 4%-20% CRCs[6,10-14], with the vast majority being the V600E hotspot mutation. Although some previous studies have shown that *BRAF* mutations confer poorer prognosis in CRC[10-12], others have not[6,13], probably because of associations with favorable MSI-H CRC prognosis[15-17].

Although genetic background and geographical factors may influence mutation frequency and prognosis, most reports are from Western countries; less data are available regarding the prognostic role of *KRAS* and *BRAF* mutations in Asian populations. Two independent studies from Taiwan and Japan have been published recently. However, both had a small sample size and heterogeneous cohorts including metastatic disease; the study from Taiwan did not examine MSI status[14,18]. Hence, a large homogenous cohort with MSI status is essential for assessing the prognostic value of various clinical or molecular variables in CRC. Here, we clarified associations of *KRAS* and *BRAF* mutations and MSI status with survival outcomes in a larger Japanese cohort of patients with curatively resected CRC.

MATERIALS AND METHODS

Patients and tissue samples

A total of 813 consecutive stage Ⅰ-Ⅲ CRC patients undergoing curative resection at Saitama Cancer Center between July 1999 and May 2006 were included. Written informed consent was obtained from all patients. Patients with the following conditions were excluded: (1) history of radiotherapy or chemotherapy preoperatively; (2) inflammatory bowel disease; or (3) history of familial adenomatous polyposis. Pathological staging was performed according to the tumor, node, and metastasis (TNM) classification system (6th edition)[19]. CRCs were typically divided into 3 types: rectum, distal colon (splenic flexure and descending and sigmoid colon), and proximal colon (cecum and ascending and transverse colon). Adjuvant chemotherapy was administered to 40% (129/322) and 76% (232/307) of stage Ⅱ and Ⅲ CRC patients, respectively. Among 361 patients treated with adjuvant chemotherapy, only 10 patients received combination chemotherapy with 5-fluorouracil, leucovorin, and oxaliplatin, while remaining were treated with single-agent fluoropyrimidines. Patients were followed-up until death or February 2012, whichever came first. We obtained approval from the Ethics Committee of Saitama Cancer Center.

Genomic DNA extraction and KRAS and BRAF mutation analysis

Primary CRCs and paired healthy colorectal mucosa obtained perioperatively were immediately frozen at -80 ℃ until analysis. Genomic DNA was extracted

from fresh frozen specimens using the standard phenol-chloroform extraction method. Exons 2 and 3 of *KRAS* were examined for mutations by denaturing gradient gel electrophoresis, as described previously[20]. The *BRAF* V600E mutation was detected using PCR and restriction enzyme digestion, as described previously[21].

MSI analysis

MSI analysis was performed using fluorescence-based PCR, as described previously[22]. Five Bethesda markers BAT25, BAT26, D5S346, D2S123, and D17S250 were used to classify tumor MSI status. MSI status was graded as MSI-H with 2 or more unstable markers, MSI-low (MSI-L) with only 1 unstable marker, and microsatellite-stable (MSS) with no unstable marker. MSI-positive markers were re-examined at least twice to confirm the result.

Statistical analysis

The aim of this study was to evaluate the impact of *KRAS*/*BRAF* mutations on prognosis in patients with resected CRC. Prognosis was evaluated according to 2 measures: overall survival (OS) and disease-free survival (DFS). OS was defined as the interval from the date of resection until death due to any cause or until the censor date of February 1, 2012. DFS was defined as the time from the date of resection to tumor recurrence, occurrence of a new primary colorectal tumor, or death due to any cause. Survival probability was estimated using the Kaplan-Meier method and compared using the log-rank test. Cox proportional hazards models were used to estimate uni- and multivariate adjusted hazard ratios for DFS and OS according to mutation status. Factors for which the multivariate models were adjusted are age (≥ 65 *vs* < 65), gender (male *vs* female), tumor stage (Ⅲ *vs* Ⅱ *vs* Ⅰ), adjuvant chemotherapy (Yes *vs* No), and status of MSI and *BRAF* or *KRAS* mutations (Yes *vs* No). To further evaluate the potential heterogeneity of the impact of *KRAS* and *BRAF* mutations according to MSI status and other covariates [age (≥ 65 *vs* < 65), gender (male *vs* female), tumor location (distal/rectum *vs* proximal), and stage (Ⅲ *vs* Ⅰ/Ⅱ)], we tested the models that included interaction terms, cross-products of gene mutation status, and another variable of interest in a multivariate Cox model. The likelihood ratio test was performed to determine the significance of the results.

Clinicopathological factor distribution according to gene mutation status was assessed using the χ^2 or Fisher's exact tests for categorical variables, when appropriate, and Student's *t*-test for continuous variables. All statistical analyses were performed using Dr. SPSS Ⅱ software (SPSS Japan Inc., Tokyo, Japan); 2-sided $P < 0.05$ was considered statistically significant.

RESULTS

Clinicopathological characteristics of KRAS and BRAF mutant tumors

Patient characteristics according to *KRAS* or *BRAF* status are summarized in Table 1. MSI status was determined in all cases, whereas mutation status was not determined in 1 case for *KRAS* and 2 for *BRAF*. *KRAS* or *BRAF* mutations were detected in 38% (312/812) and 5% (40/811) of cases, respectively. Only 1 patient harbored *KRAS* and *BRAF* mutations. *KRAS* mutations were more frequent in females than in males (43% *vs* 35%, $P = 0.02$). *BRAF* mutations were significantly more frequent in females than in males (7% *vs* 3%, $P = 0.006$), proximal than in distal or rectal tumors (13% *vs* 1% *vs* 2%, $P < 0.001$), mucinous or poorly differentiated tumors than in moderately or well-differentiated tumors (17% *vs* 4%, $P < 0.001$), and MSI-H tumors than in MSS/MSI-L tumors (36% *vs* 2%, $P < 0.001$).

Survival analysis

The median follow-up time was 87.7 mo (range: 13-148 mo). Based on univariate Cox proportional hazard analysis results (Table 2), greater age (≥ 65), male gender, advanced TNM stage, and presence of *KRAS* mutations were significantly associated with poor prognosis for DFS and OS. For *KRAS* mutant *vs KRAS* wild-type tumors, 5-year DFS was 71% *vs* 77% (log-rank $P = 0.02$; Figure 1A); 5-year OS was 80% *vs* 84%, respectively (log-rank $P = 0.01$; Figure 1B). Presence of *BRAF* mutations was not significantly associated with poorer DFS and OS in the entire cohort. For *BRAF* mutant *vs* wild-type tumors, 5-year DFS was 70% *vs* 75% (log-rank $P = 0.23$; Figure 1C); 5-year OS was 77% *vs* 83% (log-rank $P = 0.11$; Figure 1D), respectively.

In multivariate analysis, adjusting for potential prognostic variables, *KRAS* retained its prognostic impact on DFS (HR = 1.35; 95%CI: 1.03-1.75) and OS (HR = 1.46; 95%CI: 1.09-1.97; Table 3). Presence of *BRAF* mutations was significantly associated with poorer DFS (HR = 2.20; 95%CI: 1.19-4.06) and OS (HR = 2.30; 95%CI: 1.15-4.71) after adjustment (Table 3).

Survival analysis stratified by MSI status

Given the potential prognostic effect of MSI status, we evaluated interactions of *KRAS* or *BRAF* mutations with MSI status (Table 4). The effect of *KRAS* mutations on DFS and OS was limited to patients with MSS/MSI-L tumors (HR = 1.37; 95%CI: 1.05-1.80; HR = 1.49; 95%CI: 1.10-2.02, respectively); however, the *KRAS* by MSI interaction test was not significant ($P = 0.95$ and 0.70, respectively). *BRAF* mutations were significantly associated with reduced OS (HR = 2.74; 95%CI: 1.19-6.30) in MSS/MSI-L, but not MSI-H, tumors.

Table 1 Patient characteristics according to *BRAF* or *KRAS* status *n* (%)

Characteristics	KRAS status			BRAF status		
	Wild-type *n* = 500	Mutant *n* = 312	*P* value	Wild-type *n* = 771	Mutant *n* = 40	*P* value
Age (yr)			0.11			0.40
mean ± SD	63.5 ± 10.3	64.7 ± 10.3		63.9 ± 10.3	65.4 ± 11.6	
Gender			0.02			0.006
Male	308 (62)	166 (53)		459 (60)	15 (38)	
Female	192 (38)	146 (47)		312 (40)	25 (63)	
Tumor location			0.37			< 0.001
Proximal	134 (27)	98 (31)		201 (26)	31 (78)	
Distal	213 (43)	125 (40)		332 (43)	5 (13)	
Rectum	153 (31)	89 (29)		238 (31)	4 (10)	
Histological grade			0.24			< 0.001
Well/moderate	472 (94)	288 (92)		728 (94)	31 (78)	
Poor/mucinous	28 (6)	24 (8)		43 (6)	9 (23)	
T stage			0.12			0.89
1	52 (10)	31 (10)		79 (10)	4 (10)	
2	106 (21)	46 (15)		145 (19)	7 (18)	
3	286 (57)	200 (64)		462 (60)	23 (58)	
4	56 (11)	35 (11)		85 (11)	6 (15)	
LN metastasis			0.18			0.96
Yes	180 (36)	127 (41)		292 (38)	15 (38)	
No	320 (64)	185 (59)		479 (62)	25 (63)	
TNM stage			0.09			0.92
I	125 (25)	58 (19)		173 (22)	10 (25)	
II	195 (39)	127 (41)		306 (40)	15 (38)	
III	180 (36)	127 (41)		292 (38)	15 (38)	
Adjuvant chemotherapy			0.44			0.57
Yes	217 (43)	144 (46)		344 (45)	16 (40)	
No	283 (57)	168 (54)		427 (55)	24 (60)	
MSI status			0.33			< 0.001
MSS/MSI-L	455 (91)	290 (93)		728 (94)	16 (40)	
MSI-H	45 (9)	22 (7)		43 (6)	24 (60)	

SD: Standard deviation; LN: Lymph node; TNM: Tumor-Node-Metastasis; MSI: Microsatellite instability; MSS: Microsatellite stable; MSI-L: Microsatellite instability-low; MSI-H: Microsatellite instability-high.

Table 2 Univariate prognostic analysis of disease-free survival and overall survival

Characteristics	Disease-free survival		Overall survival	
	HR	95%CI	HR	95%CI
Age (yr)				
< 65	1	Reference	1	Reference
≥ 65	1.73	1.35-2.28	2.21	1.64-2.98
Gender				
Female	1	Reference	1	Reference
Male	1.57	1.20-2.06	1.57	1.16-2.13
Tumor location				
Proximal	1	Reference	1	Reference
Distal	0.92	0.67-1.25	0.9	0.64-1.26
Rectum	1.17	0.85-1.62	0.97	0.67-1.40
Histological grade				
Well/moderate	1	Reference	1	Reference
Poor/mucinous	1.53	0.97-2.42	1.43	0.84-2.42
AJCC stage				
I	1	Reference	1	Reference
II	2.6	1.61-4.19	2.26	1.36-3.75
III	4.68	2.95-7.42	3.49	2.14-5.70
Adjuvant chemotherapy				
No	1	Reference	1	Reference
Yes	1.24	0.96-1.60	1.29	1.10-1.51
MSI				
MSS/MSI-L	1	Reference	1	Reference
MSI-H	0.71	0.42-1.20	0.92	0.54-1.59
KRAS				
Wild-type	1	Reference	1	Reference
Mutant	1.35	1.04-1.74	1.44	1.08-1.92
BRAF				
Wild-type	1	Reference	1	Reference
Mutant	1.38	0.82-2.32	1.57	0.90-2.76

HR: Hazard ratio; CI: Confidence interval; MSI: Microsatellite instability; MSS: Microsatellite stable; MSI-L: Microsatellite instability-low; MSI-H: Microsatellite instability-high.

However, the *BRAF* by MSI interaction test did not reach statistical significance ($P = 0.44$).

Survival analysis stratified by other potential variables

We also analyzed the prognostic value of *KRAS* and *BRAF* mutations for OS across strata of other potential prognostic factors (Figure 2). The prognostic effect of *KRAS* mutations appeared to be consistent across potential variables, and interactions between *KRAS* status and these factors were not significant. In contrast, *BRAF* mutations were significantly associated with poor OS in stage III, but not stage I-II, disease. Interactions between *BRAF* status and TNM stage showed suggestive statistical significance

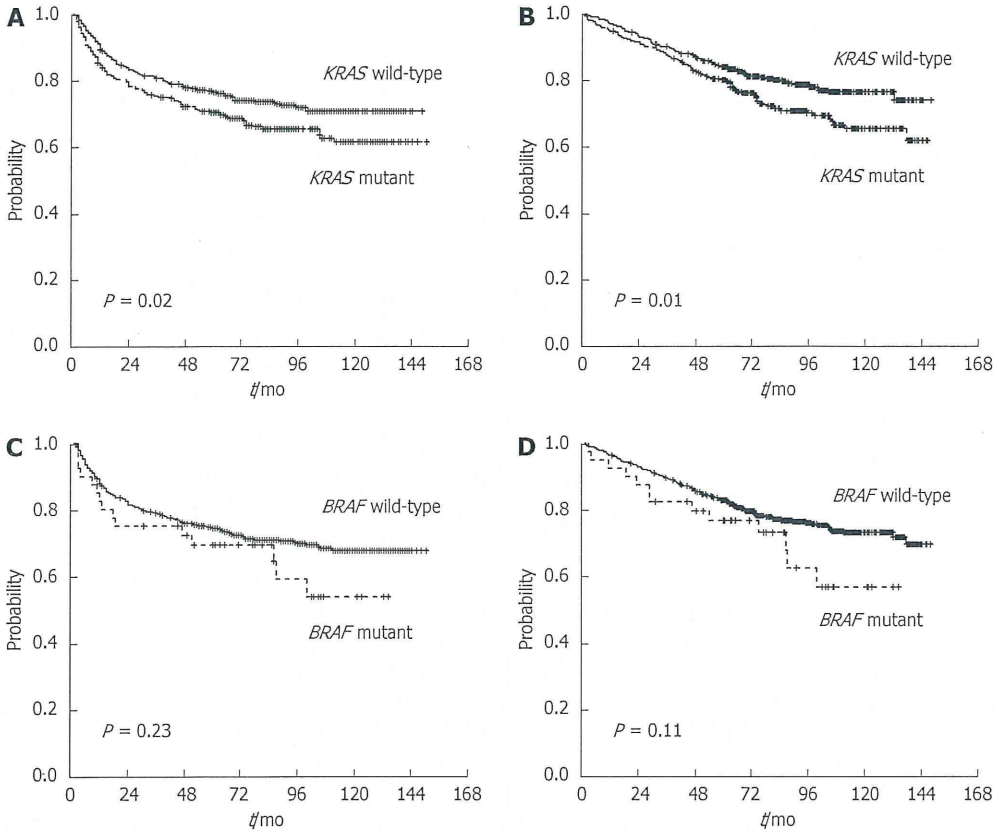

Figure 1 Kaplan-Meier curves for disease-free survival and overall survival according to *KRAS* or *BRAF* status. A: Disease-free survival (DFS) according to *KRAS* status; B: Overall survival (OS) according to *KRAS* status; C: DFS according to *BRAF* status; D: OS according to *BRAF* status.

Table 3 Prognostic effects of microsatellite instability, *KRAS*, and *BRAF* status in Cox proportional models

	Disease-free survival[1]		Overall survival[1]	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value
MSI				
MSS/MSI-L	1 (reference)	0.14	1 (reference)	0.53
MSI-H	0.64 (0.35-1.16)		0.81 (0.42-1.56)	
KRAS				
Wild-type	1 (reference)	0.03	1 (reference)	0.01
Mutant	1.35 (1.03-1.75)		1.46 (1.09-1.97)	
BRAF				
Wild-type	1 (reference)	0.01	1 (reference)	0.02
Mutant	2.20 (1.19-4.06)		2.30 (1.15-4.71)	

[1]Covariates include age (< 65 or ≥ 65), gender, AJCC stage (Ⅰ/Ⅱ/Ⅲ), adjuvant chemotherapy (Yes/No), and MSI, *KRAS*, and *BRAF* status. CI: Confidence interval; MSI: Microsatellite instability; MSS: Microsatellite stable; MSI-L: Microsatellite instability-low; MSI-H: Microsatellite instability-high.

Table 4 Prognostic Effects of *KRAS* and *BRAF* mutations according to microsatellite instability status

	KRAS		*BRAF*	
	HR (95%CI)	*P* value	HR (95%CI)	*P* value
DFS[1]				
MSS/MSI-L	1.37 (1.05-1.80)	0.95	2.06 (0.96-4.43)	0.91
MSI-H	1.34 (0.34-5.24)		2.46 (0.49-12.4)	
OS[1]				
MSS/MSI-L	1.49 (1.10-2.02)	0.70	2.74 (1.19-6.30)	0.44
MSI-H	1.39 (0.33-5.78)		1.18 (0.23-6.02)	

[1]Covariates include age, gender, AJCC stage (Ⅰ-Ⅱ/Ⅲ), adjuvant chemotherapy, and *KRAS* and *BRAF* status. HR: Hazard ratio; CI: Confidence interval; DFS: Disease-free survival; OS: Overall survival; MSI: Microsatellite instability; MSS: Microsatellite stable; MSI-L: Microsatellite instability-low; MSI-H: Microsatellite instability-high.

($P = 0.10$).

DISCUSSION

To our knowledge, this is the largest study to assess the prognostic value of *KRAS* and *BRAF* mutations for survival outcomes in CRC patients in Asian populations. Tumor specimens were prospectively collected from patients with curatively resected CRC (stage Ⅰ-Ⅲ); *KRAS* and *BRAF* mutations and MSI status were analyzed using a consistent methodology at a single institution. *KRAS* and *BRAF* mutations were associated with poor prognosis, independent of MSI status.

Many studies have examined associations of *KRAS* mutations with various clinical features, with no consistent results[5-8]. *KRAS* mutations were more frequent in females; however, these mutations were not associated with any other clinical variable.

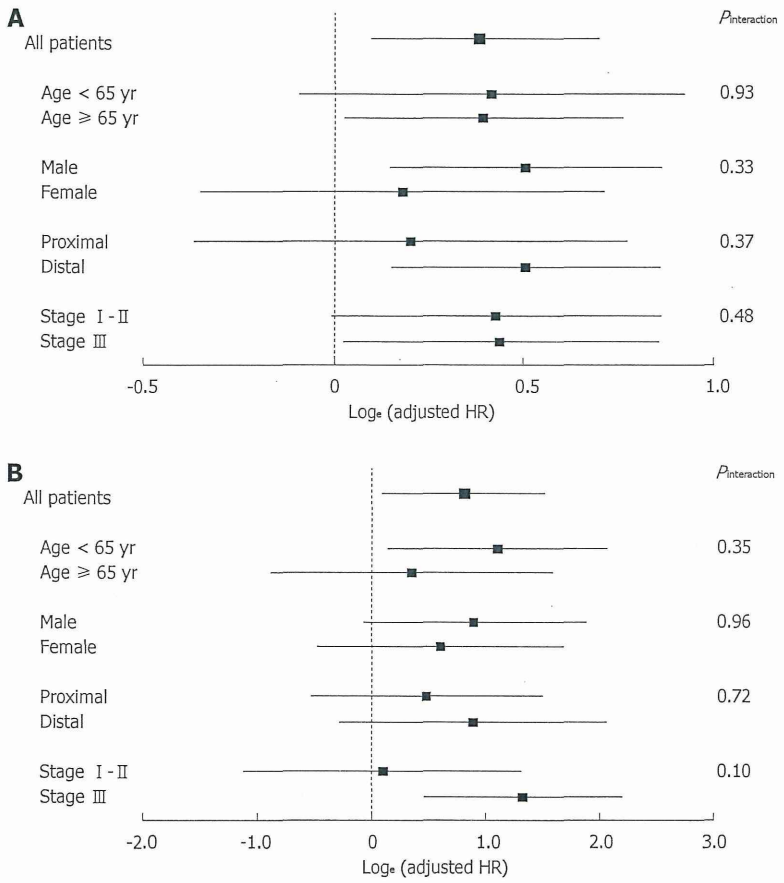

Figure 2 Stratified analysis of *KRAS* or *BRAF* status and overall survival. Loge [adjusted hazard ratio (HR)] and 95%CI for *BRAF* and *KRAS* mutant tumors (*vs* wild-type tumors) in various strata are shown. A: *KRAS* mutant tumors; B: *BRAF* mutant tumors.

Similarly, Watanabe *et al*[5] found relationships of *KRAS* mutations with the female gender and older age. In contrast, the Kirsten Ras Colorectal Cancer Collaborative Group study (RASCAL) demonstrated that *KRAS* mutations were associated with histological grade but no other variables[8]. In analysis of the PETACC-3 trial, Roth *et al*[6] reported associations of *KRAS* mutations with histological grade and tumor location but not gender. Such inconsistencies may be attributed to differences in the distribution of age, race, stage, or other factors among subject groups.

Currently, no convincing evidence exists that *KRAS* mutations are independent prognostic factors in CRC. In a Taiwanese study by Liou *et al*[14], KRAS mutations were not associated with inferior OS; however, the magnitude of multivariate HR (HR = 1.61; 95%CI: 0.91-2.84) was of the same order as that in the present study. A study from Japan revealed that the prognostic impact of *KRAS* mutations on recurrence-free survival was limited in patients with stage Ⅱ CRC, and the association of *KRAS* mutations with OS was not observed[18]. Both studies had a small sample size and heterogeneous cohorts, including stage Ⅳ disease. In the large homogeneous cohort in this study, we found significant association of *KRAS* mutations with inferior DFS and OS. Because we previously found no difference in survival outcomes among different *KRAS* mutations, including those in exons 2, 3, and 4[23], prognostic analyses of specific codons for these mutations were not performed in the present study. Similarly, the RASCAL study indicated that *KRAS* mutations resulted in overall poorer prognosis[8], whereas subsequent analysis (RASCAL Ⅱ) showed that only the glycine to valine substitution in codon 12 (G12V) was associated with poor prognosis in patients with Dukes' C disease[24]. Furthermore, recent randomized phase Ⅲ trial results supported *KRAS* mutations as prognostic factors; 3-year DFS ranged from 72% to 75% across treatments for *KRAS* wild-type tumors, with 65% to 67% for *KRAS* mutant tumors[25]. In contrast, in the PETACC-3 trial, no association was found between *KRAS* mutations and poorer relapse-free survival or OS[6]. Although further research of the prognostic effect of *KRAS* mutations is needed, the influence of these mutations seems to be mild across previously reported studies.

The frequency of *BRAF* mutations (5%) and

MSI-H (8%) in our cohort was lower than that in Western populations (*BRAF*: 8%-20%, MSI-H: 11%-17%)[6,9,11-13,15,16] and comparable with that in Asian populations (*BRAF*: 4%-7%, MSI-H: 6%-12%)[14,18,26]. Generally, *BRAF* mutations and MSI-H are frequently observed in females, proximal tumors, and poorly differentiated tumors. In a systematic review including 9885 CRC patients, a *BRAF* mutation was associated with a proximal tumor location, poor differentiation, and female sex[27]. Consistent with this observation, *BRAF* mutations were more frequent in proximal tumors, poorly differentiated tumors, and females. Previous Western cohorts showed more patients with proximal and poorly differentiated tumors compared with Asian cohorts, including the current cohort. Thus, the discrepancy in *BRAF* mutations and MSI-H status between Western and Asian populations may be attributed to the different distribution of patients' characteristics such as gender, tumor location, histological grade, or racial and/or environmental differences.

Most previous studies found associations of *BRAF* mutations with poorer survival[6,10-12]. In meta-analysis of 26 independent studies (11773 patients), *BRAF* mutations increased the risk of mortality in CRC patients (HR = 2.25; 95%CI: 1.82-2.83)[28]. However, this evidence is mainly based on studies in Western populations; little is known regarding the prognostic role of *BRAF* mutations in Asian populations. In a Taiwanese study[14], *BRAF* mutations were associated with reduced OS, but MSI status was not estimated. In a Japanese study, Nakanishi *et al*[18] found no such association because of the insufficient number of patients with *BRAF* mutations. In the present study with larger sample size and homogeneous cohorts, we found associations of *BRAF* mutations with poorer DFS and OS in CRC patients with stage I-Ⅲ disease, with the same order of magnitude of HR for OS as in the above meta-analysis. The prognostic effect of *BRAF* mutations on survival seems to be even stronger than that of *KRAS* mutations.

In contrast to previous reports[6,9,15-17], our analysis did not show that patients with MSI-H tumors exhibited better survival than those with MSS/MSI-L tumors. However, the number of patients with MSI-H tumors was too small to draw meaningful conclusions regarding the prognostic effect of MSI status. Therefore, additional larger studies are needed to clarify the prognostic impact of MSI status. Inconsistent results were reported regarding the prognostic effect of *BRAF* mutations according to MSI status[6,10,13]. Samowitz *et al*[10] found associations of BRAF mutations with poor survival in MSS/MSI-L, but not MSI-H tumors. Meanwhile, French *et al*[13] reported associations of *BRAF* mutations with poor survival in MSI-H tumors. In our analysis, associations of *BRAF* mutations with reduced OS were limited in MSS/MSI-L tumors. However, the *BRAF* by MSI interaction test was not significant; statistical power was considerably limited due to the small number of patients with MSI-H and *BRAF* mutant tumors. Larger studies are needed to clarify the modifying effect on the relation between *BRAF* mutations and survival outcome according to MSI status. Advantages of this study include comprehensive analysis of molecular markers using consistent methodology at a single institution, large sample size, and homogeneous cohort of Japanese patients. These results suggest that constitutive activation of the RAS/RAF/MAPK signaling pathway may be closely associated with clinical prognosis in CRC. Prognostic effects of *KRAS* and *BRAF* mutations seem to be consistent across most strata of clinical variables, while the adverse effect of *BRAF* mutations on OS may be attenuated in stage Ⅰ-Ⅱ CRC patients, with marginal statistical significance. The interaction of *BRAF* mutations with tumor stage warrants further research.

In conclusion, we found that Japanese CRC patients with *KRAS* or *BRAF* mutations have poorer survival, independent of MSI status. Additional investigations are warranted to clarify the interaction between these mutations and potential relevant factors, such as MSI status and tumor stage.

COMMENTS

Background

KRAS and *BRAF* mutations occur in 30%-40% and 4%-20% of colorectal cancers (CRCs), respectively. Microsatellite instability (MSI) is characterized by inactivation of the DNA mismatch repair system and is observed in 5%-15% of CRCs. MSI-high tumors are less likely to metastasize compared with the other phenotypes and have favorable survival outcomes. *KRAS* mutations are well known as predictive markers of resistance to epidermal growth factor receptor-targeted antibodies, and *BRAF* mutations are of current interest as a therapeutic target in metastatic CRCs. However, their prognostic value remains controversial for patients with curatively resected CRCs.

Research frontiers

Most previous studies investigating the prognostic role of *KRAS* and *BRAF* mutations in CRCs are from Western countries. Genetic background and geographical factors may influence mutation frequency and prognosis; however, few data are available regarding the prognostic role of these genetic alterations in Asian populations. Thus, clinical implications will be obtained by assessing the prognostic value of these mutations in a large cohort of CRCs in Japan, after adjustment for MSI status.

Innovations and breakthroughs

This study is the first large-scale study to demonstrate the prognostic impact of *KRAS* and *BRAF* mutations in Asian populations. After adjustment for relevant factors, including MSI, *KRAS* and *BRAF* mutations were independently associated with inferior disease-free survival and overall survival in patients with curatively resected CRCs. These findings will offer new insight into prognostic role of *KRAS* and *BRAF* mutations in CRCs.

Applications

BRAF and *KRAS* mutations may be useful as molecular markers for stratification of the clinical prognosis of curatively resected CRCs. Further investigation on whether the prognostic impact of *KRAS* and *BRAF* mutations could be modified by MSI status may provide more precise stratification of clinical outcomes in CRC.

Terminology

The protein product of the *KRAS* gene is a guanosine triphosphate/guanosine diphosphate-binding protein, and *KRAS* mutations play a key role in the development of various malignancies, including lung cancer, pancreatic cancer, and CRC. The protein product of the *BRAF* gene, a protein called B-Raf, is a serine/threonine protein kinase serving as downstream effector of the KRAS protein. *BRAF* mutations are involved in the development of many malignancies, *e.g.*, malignant melanoma, papillary thyroid cancer, and CRC.

Peer review

This is well written and illustrated paper. The authors investigate the prognostic role of *KRAS* and *BRAF* mutations after adjustment for MSI status. And they demonstrated that *KRAS* and *BRAF* mutations are associated with inferior survival, independent of MSI status in Asian colorectal cancer population. As the authors mentioned, in contrast to previous reports, their analysis did not show that patients with MSI-high tumors exhibited better survival than those with microsatellite-stable/MSI-low tumors.

REFERENCES

1 **Markowitz SD**, Bertagnolli MM. Molecular origins of cancer: Molecular basis of colorectal cancer. *N Engl J Med* 2009; **361**: 2449-2460 [PMID: 20018966 DOI: 10.1056/NEJMra0804588]

2 **Wang L**, Cunningham JM, Winters JL, Guenther JC, French AJ, Boardman LA, Burgart LJ, McDonnell SK, Schaid DJ, Thibodeau SN. BRAF mutations in colon cancer are not likely attributable to defective DNA mismatch repair. *Cancer Res* 2003; **63**: 5209-5212 [PMID: 14500346]

3 **Weisenberger DJ**, Siegmund KD, Campan M, Young J, Long TI, Faasse MA, Kang GH, Widschwendter M, Weener D, Buchanan D, Koh H, Simms L, Barker M, Leggett B, Levine J, Kim M, French AJ, Thibodeau SN, Jass J, Haile R, Laird PW. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. *Nat Genet* 2006; **38**: 787-793 [PMID: 16804544 DOI: 10.1038/ng1834]

4 **Yagi K**, Akagi K, Hayashi H, Nagae G, Tsuji S, Isagawa T, Midorikawa Y, Nishimura Y, Sakamoto H, Seto Y, Aburatani H, Kaneda A. Three DNA methylation epigenotypes in human colorectal cancer. *Clin Cancer Res* 2010; **16**: 21-33 [PMID: 20028768 DOI: 10.1158/1078-0432.CCR-09-2006]

5 **Watanabe T**, Yoshino T, Uetake H, Yamazaki K, Ishiguro M, Kurokawa T, Saijo N, Ohashi Y, Sugihara K. KRAS mutational status in Japanese patients with colorectal cancer: results from a nationwide, multicenter, cross-sectional study. *Jpn J Clin Oncol* 2013; **43**: 706-712 [PMID: 23657052 DOI: 10.1093/jjco/hyt062]

6 **Roth AD**, Tejpar S, Delorenzi M, Yan P, Fiocca R, Klingbiel D, Dietrich D, Biesmans B, Bodoky G, Barone C, Aranda E, Nordlinger B, Cisar L, Labianca R, Cunningham D, Van Cutsem E, Bosman F. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. *J Clin Oncol* 2010; **28**: 466-474 [PMID: 20008640 DOI: 10.1200/JCO.2009.23.3452]

7 **Samowitz WS**, Curtin K, Schaffer D, Robertson M, Leppert M, Slattery ML. Relationship of Ki-ras mutations in colon cancers to tumor location, stage, and survival: a population-based study. *Cancer Epidemiol Biomarkers Prev* 2000; **9**: 1193-1197 [PMID: 11097226]

8 **Andreyev HJ**, Norman AR, Cunningham D, Oates JR, Clarke PA. Kirsten ras mutations in patients with colorectal cancer: the multicenter "RASCAL" study. *J Natl Cancer Inst* 1998; **90**: 675-684 [PMID: 9586664]

9 **Hutchins G**, Southward K, Handley K, Magill L, Beaumont C, Stahlschmidt J, Richman S, Chambers P, Seymour M, Kerr D, Gray R, Quirke P. Value of mismatch repair, KRAS, and BRAF mutations in predicting recurrence and benefits from chemotherapy in colorectal cancer. *J Clin Oncol* 2011; **29**: 1261-1270 [PMID: 21383284 DOI: 10.1200/JCO.2010.30.1366]

10 **Samowitz WS**, Sweeney C, Herrick J, Albertsen H, Levin TR, Murtaugh MA, Wolff RK, Slattery ML. Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. *Cancer Res* 2005; **65**: 6063-6069 [PMID: 16024606 DOI: 10.1158/0008-5472.CAN-05-0404]

11 **Fariña-Sarasqueta A**, van Lijnschoten G, Moerland E, Creemers GJ, Lemmens VE, Rutten HJ, van den Brule AJ. The BRAF V600E mutation is an independent prognostic factor for survival in stage II and stage III colon cancer patients. *Ann Oncol* 2010; **21**: 2396-2402 [PMID: 20501503 DOI: 10.1093/annonc/mdq258]

12 **Ogino S**, Shima K, Meyerhardt JA, McCleary NJ, Ng K, Hollis D, Saltz LB, Mayer RJ, Schaefer P, Whittom R, Hantel A, Benson AB, Spiegelman D, Goldberg RM, Bertagnolli MM, Fuchs CS. Predictive and prognostic roles of BRAF mutation in stage III colon cancer: results from intergroup trial CALGB 89803. *Clin Cancer Res* 2012; **18**: 890-900 [PMID: 22147942 DOI: 10.1158/1078-0432.CCR-11-2246]

13 **French AJ**, Sargent DJ, Burgart LJ, Foster NR, Kabat BF, Goldberg R, Shepherd L, Windschitl HE, Thibodeau SN. Prognostic significance of defective mismatch repair and BRAF V600E in patients with colon cancer. *Clin Cancer Res* 2008; **14**: 3408-3415 [PMID: 18519771 DOI: 10.1158/1078-0432.CCR-07-1489]

14 **Liou JM**, Wu MS, Shun CT, Chiu HM, Chen MJ, Chen CC, Wang HP, Lin JT, Liang JT. Mutations in BRAF correlate with poor survival of colorectal cancers in Chinese population. *Int J Colorectal Dis* 2011; **26**: 1387-1395 [PMID: 21553007 DOI: 10.1007/s00384-011-1229-1]

15 **Sinicrope FA**, Foster NR, Thibodeau SN, Marsoni S, Monges G, Labianca R, Kim GP, Yothers G, Allegra C, Moore MJ, Gallinger S, Sargent DJ. DNA mismatch repair status and colon cancer recurrence and survival in clinical trials of 5-fluorouracil-based adjuvant therapy. *J Natl Cancer Inst* 2011; **103**: 863-875 [PMID: 21597022 DOI: 10.1093/jnci/djr153]

16 **Ribic CM**, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, Hamilton SR, Laurent-Puig P, Gryfe R, Shepherd LE, Tu D, Redston M, Gallinger S. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. *N Engl J Med* 2003; **349**: 247-257 [PMID: 12867608 DOI: 10.1056/NEJMoa022289]

17 **Popat S**, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol* 2005; **23**: 609-618 [PMID: 15659508 DOI: 10.1200/JCO.2005.01.086]

18 **Nakanishi R**, Harada J, Tuul M, Zhao Y, Ando K, Saeki H, Oki E, Ohga T, Kitao H, Kakeji Y, Maehara Y. Prognostic relevance of KRAS and BRAF mutations in Japanese patients with colorectal cancer. *Int J Clin Oncol* 2013; **18**: 1042-1048 [PMID: 23188063 DOI: 10.1007/s10147-012-0501-x]

19 **Sobin LH**, Wittekind C. TNM Classification of Malignant Tumors, 6th edition. New York: Wiley-Liss, 2002

20 **Akagi K**, Uchibori R, Yamaguchi K, Kurosawa K, Tanaka Y, Kozu T. Characterization of a novel oncogenic K-ras mutation in colon cancer. *Biochem Biophys Res Commun* 2007; **352**: 728-732 [PMID: 17150185 DOI: 10.1016/j.bbrc.2006.11.091]

21 **Asaka S**, Arai Y, Nishimura Y, Yamaguchi K, Ishikubo T, Yatsuoka T, Tanaka Y, Akagi K. Microsatellite instability-low colorectal cancer acquires a KRAS mutation during the progression from Dukes' A to Dukes' B. *Carcinogenesis* 2009; **30**: 494-499 [PMID: 19147861 DOI: 10.1093/carcin/bgp017]

22 **Ishikubo T**, Nishimura Y, Yamaguchi K, Khansuwan U, Arai Y, Kobayashi T, Ohkura Y, Hashiguchi Y, Tanaka Y, Akagi K. The clinical features of rectal cancers with high-frequency microsatellite instability (MSI-H) in Japanese males. *Cancer Lett* 2004; **216**: 55-62 [PMID: 15500949 DOI: 10.1016/j.canlet.2004.07.017]

23 **Ogura T**, Kakuta M, Yatsuoka T, Nishimura Y, Sakamoto H, Yamaguchi K, Tanabe M, Tanaka Y, Akagi K. Clinicopathological characteristics and prognostic impact of colorectal cancers with NRAS mutations. *Oncol Rep* 2014; **32**: 50-56 [PMID: 24806883 DOI: 10.3892/or.2014.3165]

24 **Andreyev HJ**, Norman AR, Cunningham D, Oates J, Dix BR, Iacopetta BJ, Young J, Walsh T, Ward R, Hawkins N, Beranek M,

Jandik P, Benamouzig R, Jullian E, Laurent-Puig P, Olschwang S, Muller O, Hoffmann I, Rabes HM, Zietz C, Troungos C, Valavanis C, Yuen ST, Ho JW, Croke CT, O'Donoghue DP, Giaretti W, Rapallo A, Russo A, Bazan V, Tanaka M, Omura K, Azuma T, Ohkusa T, Fujimori T, Ono Y, Pauly M, Faber C, Glaesener R, de Goeij AF, Arends JW, Andersen SN, Lövig T, Breivik J, Gaudernack G, Clausen OP, De Angelis PD, Meling GI, Rognum TO, Smith R, Goh HS, Font A, Rosell R, Sun XF, Zhang H, Benhattar J, Losi L, Lee JQ, Wang ST, Clarke PA, Bell S, Quirke P, Bubb VJ, Piris J, Cruickshank NR, Morton D, Fox JC, Al-Mulla F, Lees N, Hall CN, Snary D, Wilkinson K, Dillon D, Costa J, Pricolo VE, Finkelstein SD, Thebo JS, Senagore AJ, Halter SA, Wadler S, Malik S, Krtolica K, Urosevic N. Kirsten ras mutations in patients with colorectal cancer: the 'RASCAL II' study. *Br J Cancer* 2001; **85**: 692-696 [PMID: 11531254 DOI: 10.1054/bjoc.2001.1964]

25 **Alberts SR**, Sargent DJ, Nair S, Mahoney MR, Mooney M, Thibodeau SN, Smyrk TC, Sinicrope FA, Chan E, Gill S, Kahlenberg MS, Shields AF, Quesenberry JT, Webb TA, Farr GH, Pockaj BA, Grothey A, Goldberg RM. Effect of oxaliplatin, fluorouracil, and leucovorin with or without cetuximab on survival among patients with resected stage III colon cancer: a randomized trial. *JAMA* 2012; **307**: 1383-1393 [PMID: 22474202 DOI: 10.1001/jama.2012.385]

26 **Lin CH**, Lin JK, Chang SC, Chang YH, Chang HM, Liu JH, Li LH, Chen YT, Tsai SF, Chen WS. Molecular profile and copy number analysis of sporadic colorectal cancer in Taiwan. *J Biomed Sci* 2011; **18**: 36 [PMID: 21645411 DOI: 10.1186/1423-0127-18-36]

27 **Clancy C**, Burke JP, Kalady MF, Coffey JC. BRAF mutation is associated with distinct clinicopathological characteristics in colorectal cancer: a systematic review and meta-analysis. *Colorectal Dis* 2013; **15**: e711-e718 [PMID: 24112392 DOI: 10.1111/codi.12427]

28 **Safaee Ardekani G**, Jafarnejad SM, Tan L, Saeedi A, Li G. The prognostic value of BRAF mutation in colorectal cancer and melanoma: a systematic review and meta-analysis. *PLoS One* 2012; **7**: e47054 [PMID: 23056577 DOI: 10.1371/journal.pone.0047054]

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Combined Microsatellite Instability and *BRAF* Gene Status As Biomarkers for Adjuvant Chemotherapy in Stage III Colorectal Cancer

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Background: The clinical relevance of combined microsatellite instability (MSI) and *BRAF* status for adjuvant treatment in stage III colorectal cancer (CRC) remains elusive.
Methods: In 405 patients with curatively resected stage III CRC, the prognostic value of combined MSI and *BRAF* status was assessed in four groups, as follows: high-levels of microsatellite instability (MSI-H) and *BRAF*-wild type, MSI-H and *BRAF*-mutation, microsatellite stable (MSS) and *BRAF*-wild type, and MSS and *BRAF*-mutation.
Results: Combined MSI and *BRAF* status provided significant prognostic stratification of disease-free survival (DFS), and was independently associated with worse DFS. The MSI-H and *BRAF*-wild type group had similar outcomes to stage II CRC patients, despite no benefit from 5-FU monotherapy. Further, patients in the MSS and *BRAF*-wild type group with stage IIIA CRC had favorable outcomes to 5-FU monotherapy, similar to those with stage II CRC. In contrast, 5-FU monotherapy was insufficient among patients in the MSS and *BRAF*-wild type group with stage IIIB or IIIC CRC or patients in the MSS and *BRAF*-mutation group with stage III CRC.
Conclusions: The combination of MSI and *BRAF* status serves as both a prognostic and predictive marker and may provide much-needed guidance during the planning of therapeutic strategies.
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KEY WORDS: MSI; *BRAF*; colorectal cancer; adjuvant chemotherapy

INTRODUCTION

5-fluorouracil (5-FU)-based adjuvant chemotherapy in combination with oxaliplatin (L-OHP) is the current worldwide standard of care for patients with stage III colorectal cancer (CRC) [1–3]. However, this combination therapy has carries significant monetary costs as well as patient inconvenience and toxicity, particularly L-OHP-induced cumulative dose-dependent neurotoxicity. Therefore, decision regarding adjuvant treatment must be based on thorough discussion with the patient on an individual basis taking into account patient characteristics and cancer features [4].

The DNA mismatch repair (MMR) system has a DNA damage sensor function, which induces apoptosis via p53 action or G2 cell cycle arrest in response to 5-FU-modified DNA damage [5]. Microsatellite instability (MSI) status is the molecular fingerprint of the MMR system, and the high-levels of MSI (MSI-H) phenotype exhibits favorable outcomes and a lack of survival benefit from 5-FU-based adjuvant chemotherapy in contrast to the microsatellite stable (MSS) phenotype, indicating clinical relevance for use of 5-FU-based chemotherapy [6–10].

BRAF is an essential component of the Ras/Raf/MAPK signaling cascade and is commonly activated by the *BRAF* V600E mutation [11,12]. *BRAF* mutations frequently occur in sporadic CRC with MSI-H phenotype [13]. In contrast to the MSI-H phenotype, *BRAF* mutation has been associated with a poor clinical outcome [14–17], and several studies have suggested the use of combined MSI and *BRAF* status evaluation as a molecular marker for prognostic risk in adjuvant treatment [14–18]. However, the majority of these previous studies analyzed populations including various disease stages, despite routine adjuvant chemotherapy only being recommended in stage III CRC by the National Comprehensive Cancer Network (NCCN) guidelines [19].

The American Joint Committee on Cancer (AJCC) TNM staging system is regarded as the most clinically useful prognostic marker, stratifying patients with stage III CRC into three subgroups with different outcomes: stage IIIA, stage IIIB, and stage IIIC [20,21]. However, it remains to be determined whether or not the combination of MSI and *BRAF* status can confer additional markers that are both prognostic and predictive of a response to therapy within each subgroup of stage III CRC. Here, we examined the clinical relevance of the MSI and *BRAF* combination according to the three subgroups of AJCC TNM stage III CRC and assessed the clinical potential for the establishment of personalized therapeutic strategies in adjuvant chemotherapy.

MATERIALS AND METHODS

Patient Population

We evaluated a series of 405 consecutive patients with pathologically confirmed stage III CRC and the available tumor specimens of those who underwent curative surgical resection with regional lymph nodes dissection at Saitama Cancer Center from May 2001 to December 2011. In addition, 342 stage II CRC patients were included to compare outcomes with stage III. Exclusion criteria were active concomitant

Conflict of Interest: None.

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