Clinical and prognostic features of patients with detailed *RAS/BRAF*-mutant colorectal cancer in Japan

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Abstract

Background: *RAS/BRAF*^{V600E} mutations are the most remarkable oncogenic driver mutations in colorectal cancer (CRC) and play an important role in treatment selection. No data are available regarding the clinical and prognostic features of patients with detailed *RAS/BRAF*^{V600E}-mutant metastatic CRC (mCRC) in Japan.

Methods: Chemotherapy-naïve patients with mCRC were included in this study between August 2018 and July 2019. Tumor samples were collected, and *RAS/BRAF*^{V600E} status was investigated. *RAS/BRAF*^{V600E} status was examined using a MEBGEN RASKET-B kit and polymerase chain reaction reverse sequence-specific oligonucleotide method.

Results: *RAS/BRAF*^{V600E} mutations were detected in 54% of cases (*KRAS* codon 12 = 26%; *KRAS* codon 13 = 11%; minor *RAS* [non-*KRAS* codon 12 and non-*KRAS* codon 13] = 10%; and *BRAF*^{V600E} = 7%). *BRAF*^{V600E}-mutant CRC mainly existed in the right colon, whereas minor *RAS*-mutant CRC was predominantly present in the left colon. Minor *RAS*-mutant CRC was associated with shorter survival time than *RAS* wild-type CRC (hazard ratio [HR] = 2.45; 95% confidence interval [CI] = 0.92–6.57; *p* = 0.073) and significantly shorter overall survival than major *RAS*-mutant CRC (HR = 4.77; 95% CI = 1.44–15.77; *p* = 0.010). The *KRAS* codon 12 mutant group showed significantly longer overall survival than the minor *RAS*-mutant groups (HR = 0.06; 95% CI = 0.01–0.47; *p* < 0.00) and had extended progression-free survival compared with the codon 13 group.

Conclusions: In our multicenter study, the findings elucidated the clinical and prognostic features of patients with detailed *RAS/BRAF*^{V600E}-mutant mCRC in Japan.

Background

Colorectal cancer (CRC) is one of the most predominant malignant tumors worldwide, including in Japan. Treatment for advanced recurrent or metastatic CRC (mCRC) aims to control disease activity using anticancer drugs. Investigating actionable mutations, especially rat sarcoma (*RAS*) and rapidly accelerated fibrosarcoma (*RAF*), is imperative for drug selection in mCRC treatment. *RAS* and *RAF* mutations control various activities, such as angiogenesis, proliferation, and apoptosis, and play an important role as prognostic and predictive indicators in CRC treatment^{1–9}.

Patients with mCRC with *RAS* (*KRAS/NRAS*) mutations receive less benefit from anti-epidermal growth factor receptor (EGFR) therapy because *RAS* mutations activate downstream pathways without depending on EGFR and trigger primary resistance^{2,7,10–14}. In particular, mutations in the amino acid at position 12 in the *KRAS* protein are general; the *KRAS* p.G12C mutation accounts for ~3% of patients with CRC and is significantly associated with poor prognosis¹⁵. Although there is no adequate progress in drug development for *RAS*-mutant tumors, recently developed drugs are expected to be effective. The CodeBreak 100 trial revealed the potent antitumor effects of AMG510, a novel *KRAS* G12C inhibitor, against *KRAS* G12C-mutant solid tumors, including mCRC¹⁶.

Focusing on *BRAF*^{V600E}, previous studies have shown that patients with mCRC with *BRAF* mutations have worse outcomes than those with *BRAF* wild-type^{17–19}. There have been discrepant results regarding the efficacy of anti-EGFR antibodies in *BRAF*- and *KRAS*-mutant cases^{20,21}. In the BEACON CRC trial, a novel triplet combination regimen of encorafenib (*BRAF* inhibitor), binimetinib (*MEK* inhibitor), and cetuximab (anti-EGFR antibody), or a novel doublet combination regimen of encorafenib and cetuximab showed benefits compared with the current standard therapy and is now recognized as a new standard¹⁶.

The presence or absence of *RAS/BRAF* mutations can affect anticancer therapy options. In general, patients with *RAS/BRAF* mutations have different clinical characteristics, and new therapeutic agents developed for driver mutations of these CRCs have been gaining popularity^{19,23}. However, no detailed data have been reported on the clinical and prognostic features in Asian patients, including those from Japan, with detailed *RAS/BRAF*^{V600E}-mutant mCRC. Therefore, in the present multicenter retrospective study, we aimed to determine the clinical and prognostic features of mCRC with a detailed *RAS/BRAF*^{V600E} mutation in Japan.

Methods

Patient selection and characteristics

In total, 152 patients with advanced recurrent CRC were included in the present study, for whom tissue *RAS/BRAF* testing was performed between August 2018 and July 2019. Patient tumor samples taken from primary or metastatic sites were used to investigate the *RAS/BRAF*^{V600E} mutation status. The following clinical data were collected from three institutions: age, sex, location of the primary tumor, pathological differentiation, stage and TNM grade, metastatic sites, first-line systemic chemotherapy regimen, duration and the best efficacy of first-line chemotherapy, date of confirmation of tumor growth after first-line chemotherapy, date of last consultation date, and date of death.

Analysis for *RAS/BRAF* mutation

Genomic DNA was detected in each patient using formalin-fixed paraffin-embedded tumor samples. In total, 49 *RAS/BRAF* mutations were analyzed using the MEBGEN RASKET-B kit and polymerase chain reaction reverse sequence-specific oligonucleotide method for all enrolled cases^{12,24}. The mutations included those in *KRAS* codon 12 (G12S, G12C, G12R, G12D, G12V, and G12A), *KRAS* codon 13 (G13S, G13C, G13R, G13D, G13V, and G13A), *KRAS* codon 59 (A59T and A59G), *KRAS* codon 61 (Q61K, Q61E, Q61L, Q61P, Q61R, and Q61H), *KRAS* codon 117 (K117N), *KRAS* codon 146 (A146T, A146P, and A146V), *NRAS* codon 12 (G12S, G12C, G12R, G12D, G12V, and G12A), *NRAS* codon 13 (G13S, G13C, G13R, G13D, G13V, and G13A), *NRAS* codon 59 (A59T and A59G), *NRAS* codon 61 (Q61K, Q61E, Q61L, Q61P, Q61R, and Q61H), *NRAS* codon 117 (K117N), *NRAS* codon 146 (A146T, A146P, and A146V), and *BRAF* codon 600 (V600E).

Assessment and statistical analysis

Disease assessment was usually performed every 8 ± 2 weeks using computed tomography (CT). The response was evaluated using CT images based on the Response Evaluation Criteria in Solid Tumors version 1.1. We defined overall survival (OS) as the time from enrollment in our study to the date of death for any reason or last consultation date. Progression-free survival (PFS) was defined as the time from enrollment in our study to initial disease progression or death, whichever occurred earlier. We defined the overall response rate as the percentage of patients who achieved a complete response or partial response relative to the total number of enrolled patients based on CT images. Statistical analyses were performed using SPSS statistics version 27.0, and a statistically significant difference was considered at a value of $p < 0.05$. Fisher's exact test was used to compare the characteristics of each patient. Statistical analyses of OS and PFS were performed using the Kaplan–Meier method. The log-rank test was used to compare each group, whereas Cox regression analysis was used to estimate the hazard ratio (HR) with a 95% confidence interval (CI). We also evaluated whether *RAS/BRAF*^{V600E} status was associated with OS and PFS.

Results

Patient characteristics and frequency of *RAS/BRAF*^{V600E} mutation subtypes

In total, 152 patients were investigated for *RAS/BRAF*^{V600E} status from three institutions. Table 1 shows the characteristics of the patients included in this study. The frequency of *RAS* mutations was 47% ($n = 72$), whereas that of the wild-type and *BRAF*^{V600E} mutations was 46% ($n = 70$) and 7% ($n = 10$), respectively. *KRAS* mutations were found in codon 12 in 26% of cases and codon 13 in 11% of cases; therefore, we designated *KRAS* codons 12 and 13 as the major *RAS* mutation groups. The other *RAS* (non-*KRAS* codon 12 and non-*KRAS* codon 13) mutations were designated as the minor *RAS* mutation group, which included 10% of cases ($n = 15$; Fig. 1). The locations of the primary tumors in each *RAS/BRAF* mutation are shown in Fig. 2.

[Table 1] Clinical characteristics and concomitant mutations of patients with *RAS/BRAF*^{V600E} mutant colorectal cancer.

Characteristics	Total N = 152 (%)	Major N = 57 (%)	Minor N = 15 (%)	<i>BRAF</i> ^{V600E} N = 10 (%)	Wild N = 70 (%)
Age (years), median [range]	71 [18–89]	71 [38–89]	77 [56–87]	71 [64–80]	70 [18–86]
< 75/≥75	99/53	37/20 (65/35)	5/10 (33/67)	7/3 (70/30)	50/20 (71/29)
		p = 1.000	p = 0.010	p = 1.000	p = 0.172
Sex, Male/Female	97/55 (71/29)	33/24 (58/42)	10/5 (67/33)	4/6 (40/60)	50/20 (71/29)
		p = 0.296	p = 1.000	p = 0.170	p = 0.091
Primary, Right/Left/Unknown	48/102/2 (31.5/67.1/1.3)	21/49/0 (37/63/0)	1/14/0 (7/93/0)	7/2/1 (70/20/10)	19/49/2 (27/70/3)
		p = 0.371	p = 0.038	p = 0.005	p = 0.379
Differentiation					
tub1/tub2/por/sig/others/unknown	57/70/6/2/10/7	22/29/2/0/2/2	5/9/1/0/0/0	2/5/0/0/2/1	28/27/3/2/6/4
Stage					
I/II/III/IV/unknown	3/15/37/95/2	2/9/12/34/0	0/1/4/10/0	0/0/2/7/1	1/5/19/44/1
P-value: Fisher's exact test, and the value of the comparison between this group and other groups.					

Clinicopathological characteristics of each *RAS/BRAF*^{V600E} group

We investigated the relationship between *RAS/BRAF*^{V600E} mutation rate and age (< 75 and ≥ 75 years), sex, and the location of the primary tumor (Table 1). Minor *RAS* mutations were more common in patients aged ≥ 75 years, whereas no correlation was observed between age and frequency in the other groups. We found no significant differences in sex between the groups. Minor *RAS* mutations were significantly more common in the left colon than in the right colon (p = 0.038), whereas *BRAF*^{V600E} mutations were significantly more common in the right colon than in the left colon (p = 0.005).

OS of patients with each *RAS/BRAF*^{V600E} status

Among the 152 patients, 125 received systemic chemotherapy and were investigated for OS using the Kaplan-Meier method. We analyzed the OS in each *RAS/BRAF*^{V600E} mutation group (Fig. 3). The OS in the wild-type group was longer than that in the minor and *BRAF*^{V600E} mutation groups; however, we did not observe significant differences between these groups (HR = 2.45; 95% CI = 0.92–6.57; p = 0.073 and HR = 1.11; 95% CI = 0.25–5.0; p = 0.894, respectively). The OS was significantly longer in the major *RAS* mutation group than in the minor *RAS* mutation group (HR = 4.77; 95% CI = 1.44–15.7; p = 0.01). The OS was shorter in the minor *RAS* mutation group than in the *BRAF*^{V600E} mutation group; however, we did not observe significant differences between these groups (HR = 0.48; 95% CI = 0.10–2.41; p = 0.375).

OS and PFS in each *RAS* mutation group

Among the 125 patients who received systemic chemotherapy, 57 had *RAS* mutations. We investigated OS and PFS using the Kaplan-Meier method (Figs. 4 and 5). OS was considered regardless of treatment modality; however, PFS was analyzed only in patients who were treated with doublet therapy with anti-vascular endothelial growth factor (anti-VEGF) agents as the primary treatment. OS was significantly longer in the *KRAS* codon 12 group than in the *KRAS* codon 13 group (HR = 7.84; 95% CI = 0.81–

76.2; $p = 0.04$), and PFS was longer in the *KRAS* codon 12 group than in the *KRAS* codon 13 group (HR = 3.12; 95% CI = 0.90–10.8; $p = 0.06$). The OS was shorter in the minor group than in the *KRAS* codon 12 group (HR = 0.06; 95% CI = 0.01–0.47; $p < 0.00$).

Discussion

In the present study, we investigated the frequency of *RAS/BRAF*^{V600E} mutations in 152 patients with mCRC in Japan and evaluated the association between each mutation and its clinical and pathological characteristics. We divided *RAS* mutations into the following two groups: *KRAS* codon 12 and *KRAS* codon 13 mutations as the major mutation group and other mutations as the minor mutation group. *KRAS* codon 12 and *KRAS* codon 13 mutations were observed in 26% and 11% of cases, respectively. In Japan, *KRAS* codon 12 and *KRAS* codon 13 mutations account for 29.9–34.1% and 3.8–7.7% of cases, respectively, which is consistent with the results of the present study^{23,25}. *BRAF*^{V600E} accounted for approximately 7% of cases in the present study, whereas previous studies have reported a range of 5–21% for the *BRAF*^{V600E} mutation^{18,20}. The frequency of minor *RAS*-mutant mCRC was the same as that in a previous study²⁴; however, to date, no data have been published on the frequency of minor *RAS*-mutant CRC in Asian populations, including Japanese patients.

We observed a relationship between the location of the primary lesion and distribution of *RAS/BRAF*^{V600E} status. *BRAF*^{V600E} mutations are more common in women over 60 years of age and in the right colon^{9,18,19,22}. In the present study, *BRAF*^{V600E} mutations were also more common in the right colon. There was a significant difference in the prevalence of *BRAF*^{V600E} mutations on the right side, but not with regard to age and sex, although it tended to be higher in females than in males. Thus, it was assumed that these mutations occurred by chance, and significant differences in each category became apparent as the number of cases increased. Minor *RAS* mutations were more common in the primary tumor of the left colon and in elderly patients. There are many studies on the clinical findings of major *RAS/BRAF*^{V600E}-mutant and *RAS/BRAF*^{V600E}-wild types. However, literature focusing on minor *RAS* mutations is rare, and the present study may be representative of minor *RAS* mutations.

We also investigated the relationship between prognosis and *RAS/BRAF*^{V600E} status. In general, the prognosis of wild-type *RAS* was better than that of the *RAS/BRAF*^{V600E} mutant; however, there were no significant differences in our study. Among the *RAS* mutations, the *KRAS* codon 12 mutation was associated with the best prognosis. Only the *RAS* minor mutation was associated with a shorter OS than the *RAS/BRAF*^{V600E} wild-type (HR = 2.45; 95% CI = 0.92–6.57; $p = 0.073$). Furthermore, patients with a *RAS* minor mutation had a significantly shorter OS than those with a *RAS* major mutation (HR = 4.77; 95% CI = 1.44–15.7; $p = 0.01$). Several studies have suggested a poorer prognosis for patients with *NRAS* mutations, a type of *RAS* minor mutation, than for those with *KRAS* mutations²⁶. The classification of minor *RAS* mutations has not been widely reported; however, we hypothesized that this result could be supported by the differences in OS between patients with different *RAS* mutations.

Similarly, we examined PFS for each *RAS* mutation type. PFS was limited to primary treatment, and the treatment regimen was limited to doublet therapy with anti-VEGF agents. Doublet therapy was defined as oxaliplatin-based and irinotecan-based regimens, and the anti-VEGF agents included bevacizumab, ramucirumab, and aflibercept. Under these conditions, a *KRAS* codon 13 mutation was associated with shorter PFS than *KRAS* codon 12 and *RAS* minor mutations, especially in comparison to a *KRAS* codon 12 mutation. A previous study from China showed that *KRAS* codon 13 and *BRAF*^{V600E} mutations are poor prognostic factors²⁷. Therefore, we suggest that a *KRAS* codon 13 mutation may be responsible for the shortened PFS in the Japanese population. This may be influenced by factors such as resistance to anti-VEGF agents, which warrants further study.

This study has a few limitations. First, this was a retrospective study with a relatively small sample size. Second, we did not follow up with most patients until death; therefore, follow-up data were insufficient.

Conclusion

This multicenter study revealed the clinical and prognostic features of patients with detailed *RAS/BRAF*^{V600E}-mutant mCRC in Japan. Each mutation had a different character, and minor mutations might have prognostic significance. In the present study, the *RAS* minor mutants were primarily in the left colon; to the best of our knowledge, this is the first study to reveal such findings

in a Japanese population. The prognosis of patients in the *RAS* minor mutant group was worse than that of patients in the other group. Although the present study involved a relatively small number of patients, the results provide a basis for the development of specific drugs for *RAS* minor and *RAS* major mutants, which have a poor prognosis.

Abbreviations

CRC, colorectal cancer; EGFR, epidermal growth factor receptor; mCRC, metastatic colorectal cancer; RAF, rapidly accelerated fibrosarcoma; RAS, rat sarcoma.

Declarations

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was carried out in accordance with the Helsinki Declaration and Ethical Guidelines for Clinical Studies and was approved by the institutional review boards of all three participating hospitals, Kobe City Medical Center General Hospital, Kansai Medical University Hospital, and Sano Hospital. Written informed consent was obtained from all the participating patients before entering the study.

CONSENT FOR PUBLICATION:

Not applicable.

AVAILABILITY OF DATA AND MATERIALS:

The datasets generated and/or analysed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

COMPLETING INTERESTS:

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AUTHORS' CONTRIBUTIONS:

Study concepts: SH, TI

Study design: SH, TI

Data acquisition: SH, KM, IT, MT, NH, BS, SN, YH

Data analysis and interpretation: SH, IT, SM

Statistical analysis: SM

Manuscript preparation: SH, IT

Manuscript editing: SH, IT

Manuscript review: All authors

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None.

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Figures

Figure 1

Frequencies of RAS/BRAFV600E mutation subtypes. N = 152 (major group: KRAS codon 12 and codon 13; minor group: other mutations; wild group: no mutations)

Figure 2

Frequency of RAS/BRAFV600E mutations by primary tumor site (A/C, ascending colon; T/C, transverse colon; D/C, descending colon; S/C, sigmoid colon)

Figure 3

Overall survival (OS) of patients with different subtypes of RAS/BRAFV600E mutations. Analysis of hazard ratio of OS based on RAS/BRAFV600E mutation status in patients with colorectal cancer using Cox regression analysis. P-value: Log-rank analysis.

Figure 4

Overall survival (OS) of patients with different subtypes of RAS mutations. Analysis of hazard ratio of OS based on RAS mutation status in patients with colorectal cancer using Cox regression analysis. P-value: Log-rank analysis.

Figure 5

Progression-free survival (PFS) of patients with different subtypes of RAS mutations and treated with doublet therapy with anti-vascular endothelial growth factor (anti-VEGF) agents. Analysis of PFS of hazard ratio based on RAS mutation status in patients with colorectal cancer treated with doublet therapy with anti-VEGF agents using Cox regression analysis. P-value: Log-rank analysis.