Clinical and prognostic characteristics of metastatic colorectal cancer with minor *RAS* mutations

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Abstract. Metastatic colorectal cancer (mCRC) presents notable therapeutic challenges. Rat sarcoma virus (*RAS*) mutations, including those in *KRAS* exon 2, are critical for treatment decisions; however, the role of minor *RAS* mutations (*KRAS* exons 3 and 4, and *NRAS*) remains underexplored. Because these mutations are increasingly identified in routine practice due to advances in *RAS* mutation testing that now routinely includes *KRAS* exons 3 and 4, and *NRAS*, clarifying their clinical relevance has become important for ensuring appropriate treatment selection. The present study aimed to compare the clinical and prognostic characteristics of patients with mCRC with minor *RAS* mutations to those with *KRAS* exon 2 mutations. To this end, data were retrospectively collected from patients with mCRC and *RAS* mutations between August 2018 and December 2023. Patients were grouped based on *RAS* mutation subtype: *KRAS* exon 2 or minor *RAS* mutations. *RAS* mutation testing was performed using the MEBGEN RASKET™-B kit. Clinical characteristics, tumor location, metastatic patterns and survival outcomes were analyzed. Overall survival (OS) and progression-free survival (PFS) were assessed using Kaplan-Meier survival analysis, log-rank tests and Cox proportional hazards regression models. Of 202 patients with *RAS* mutations, 170 had *KRAS* exon 2 mutations, whereas 32 exhibited minor *RAS* mutations (20 with non-exon 2 *KRAS* mutations and 12 with *NRAS* mutations). Minor *RAS* mutations were more common in left-sided CRC. No significant differences in background were observed between the two groups. Log-rank OS was comparable for patients with *KRAS* exon 2 and minor *RAS* mutations. OS and PFS with first-line bevacizumab-containing therapy were also similar between the two groups. In conclusion, the prognostic impact of minor *RAS* mutations appears to be comparable to that of *KRAS* exon 2 mutations, suggesting that the current treatment strategies for *RAS*-mutant CRC may not require modification based on these findings.

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Abbreviations: CRC, colorectal cancer; mCRC, metastatic CRC; RAS, rat sarcoma virus; RAF, rapidly accelerated fibrosarcoma; EGFR, epidermal growth factor receptor; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; CI, confidence interval; OS, overall survival; PFS, progression-free survival; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, overall response rate; MSI, microsatellite instability

Key words: CRC, *RAS* mutations, minor *RAS* mutations, *NRAS*, prognostic characteristics

Introduction

Colorectal cancer (CRC) is a leading cause of cancer-related deaths worldwide, with metastatic CRC (mCRC) posing therapeutic challenges (1). Treatment strategies often rely on targeted therapies guided by biomarkers such as rat sarcoma virus (*RAS*) and rapidly accelerated fibrosarcoma (*RAF*) mutations. These mutations influence key biological processes, including angiogenesis, cell proliferation, and apoptosis, and serve as critical prognostic markers (2-13).

KRAS is a small GTP-binding protein that plays a critical role in transmitting growth signals downstream from the epidermal growth factor receptor (EGFR). *KRAS* gene mutations are present in approximately half of CRC cases. Approximately 90% of these mutations occurring in *KRAS* exon 2 (codon 12, 13) (1). *KRAS* exon 2 mutations are the most common predictor of resistance to the anti-EGFR drugs, cetuximab and panitumumab, in patients with mCRC, given that *KRAS* mutations reduce the intrinsic GTPase activity of Ras, causing it to remain in its active, GTP-bound state.

Thus, despite EGFR inhibition, downstream proliferative signaling persists, leading to resistance (3-7,9). Subsequently, reports from the European consortium indicated that other *KRAS* mutations, including *KRAS* exons 3 (codon 59, 61) and 4 (codon 117, 146), and *NRAS* mutations, including *NRAS* exons 2 (codon 12, 13), 3 (codon 59, 61), and 4 (codon 117, 146), were also associated with resistance to anti-EGFR antibody therapy (14-21). These findings led to a consensus that all *RAS* mutations can predict resistance to anti-EGFR antibodies (8,10,11). However, *KRAS* exons 3 and 4, and *NRAS* mutations are relatively rare, and prior studies have examined only a limited number of cases. Additionally, in real-world clinical practice, mCRC with *RAS* mutations is commonly treated with chemotherapy regimens that include angiogenesis inhibitors, not anti-EGFR monoclonal antibodies. However, it remains unclear whether the prognosis in patients with *KRAS* exons 3 and 4, or *NRAS* mutations is comparable to that in patients with *KRAS* exon 2 mutations in this treatment setting.

Historically, only *KRAS* exon 2 mutations were detectable and therefore recognized as the primary *RAS* mutations associated with resistance to anti-EGFR antibody therapy. With advances in sequencing technologies, additional mutations in *KRAS* non-exon 2 regions of *KRAS* as well as *NRAS* mutations became identifiable. As a result, the definition of anti-EGFR resistance biomarkers expanded from *KRAS* exon 2 mutations alone to encompass the entire spectrum of RAS mutations. However, despite this unified classification, the biological characteristics, treatment responses, and prognostic implications of *KRAS* exon 2 mutations vs. *KRAS* non-exon 2 mutations remain insufficiently understood. Currently, patients with any *RAS* mutation are broadly categorized into a single group, although this approach may overlook meaningful heterogeneity within the *RAS*-mutated population.

Against the background this study aimed to focus on the relatively rare *KRAS* exons 3 and 4, and *NRAS* mutations to evaluate their impact on prognosis, clinical characteristics, and efficacy of angiogenesis inhibitors The study also sought to determine whether these impacts are comparable to those observed with *KRAS* exon 2 mutations. These mutations are categorized as minor RAS mutations in this study.

Patients and methods

Patient selection and characteristics. This retrospective cohort study was conducted at Osaka International Cancer Institute. Patients who underwent tissue *RAS* testing between August 2018 and December 2023 were included in this analysis. Tumor tissue samples for RAS testing were obtained as part of routine clinical care, and no additional samples were collected specifically for this study. As this was a retrospective observational study, no formal sample size calculation was performed. Instead, all eligible patients treated during the study period were included to enhance the representativeness and generalizability of the findings. Tumor tissue samples were analyzed to determine *RAS* mutation status using the MEBGEN RASKET™-B kit. Information regarding RAS mutation subtypes was extracted from existing medical records.

Patients with histologically confirmed colorectal adenocarcinoma and documented *RAS* mutations were included. Patients who had not received systemic chemotherapy for recurrent or metastatic CRC were excluded. For patients who experienced recurrence during or within 6 months of completing adjuvant chemotherapy, the start date of adjuvant chemotherapy was considered the initiation point for all statistical analyses, rather than the initiation point for mCRC treatment.

Demographic and clinical data, including age, sex, Eastern Cooperative Oncology Group (ECOG) performance status, location of the primary tumor, pathological differentiation, microsatellite instability status, metastatic disease characteristics, first-line chemotherapy regimen, and the best efficacy of first-line chemotherapy, were extracted from electronic medical records. Extracted metastatic characteristics included the number of metastatic organs and the presence or absence of liver and lung metastases. Tumor location was categorized both as right-sided (from the cecum to the transverse colon) or left-sided (from the splenic flexure to the rectum) and as colonic (from the cecum to the sigmoid colon) or rectal (rectum). *RAS* mutation subtypes were recorded and categorized into two groups: *KRAS* exon 2 mutations (codons 12 and 13) and minor *RAS* mutations (*KRAS* exons 3 and 4, and *NRAS* mutations). These categorizations allowed for further subgroup analyses based on clinical and molecular characteristics. We report the efficacy results using a data cutoff of December 2024.

RAS mutation analysis. Tumor tissues were obtained from primary or metastatic sites and preserved as formalin-fixed paraffin-embedded (FFPE) specimens. DNA was extracted from the FFPE blocks, and *RAS* mutation testing was performed using the MEBGEN RASKET™-B kit (22,23). Assays with the RASKET-B kit were performed according to the manufacturer's protocol. Briefly, this multiplex PCR-based assay was specifically designed to detect mutations in *KRAS* and *NRAS* genes across exons 2, 3, and 4. The mutations included those in *KRAS* codons 12 (G12S, G12C, G12R, G12D, G12V, and G12A), 13 (G13S, G13C, G13R, G13D, G13V, and G13A), *KRAS* codon 59 (A59T and A59G), 61 (Q61K, Q61E, Q61L, Q61P, Q61R, and Q61H), 117 (K117N), and 146 (A146T, A146P, and A146V), as well as *NRAS* codons 12 (G12S, G12C, G12R, G12D, G12V, and G12A), 13 (G13S, G13C, G13R, G13D, G13V, and G13A), 59 (A59T and A59G), 61 (Q61K, Q61E, Q61L, Q61P, Q61R, and Q61H), 117 (K117N), and 146 (A146T, A146P, and A146V). All procedures were conducted according to the manufacturer's protocol, ensuring accurate mutation identification with high sensitivity and specificity.

Assessment and statistical analysis. The primary outcome of the study was overall survival (OS), defined as the time from treatment initiation to death from any cause or the last follow-up date. The study included a comparison of OS between patients with *KRAS* exon 2 mutations and those with minor *RAS* mutations to assess differences in clinical outcomes. Patients who were alive at the end of the study period were censored at their most recent follow-up date (December 2024). Secondary outcomes included the prevalence of tumor location (analyzed as right-sided vs. left-sided and colon vs. rectum) by *RAS* mutation subtype, descriptive statistics of clinical characteristics, the relationship between metastatic disease features and survival outcomes, and progression-free survival (PFS) analysis for patients treated with bevacizumab in the first-line setting.

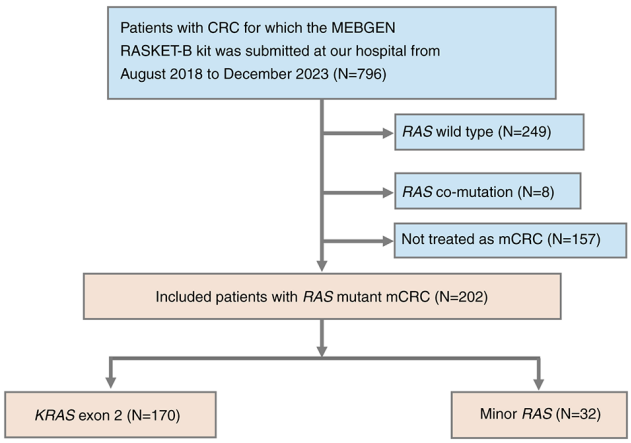

Figure 1. CONSORT diagram. CRC, colorectal cancer; mCRC, metastatic CRC; *RAS*, rat sarcoma virus.

Survival analyses were conducted using the Kaplan-Meier method to generate survival curves, with differences between groups evaluated using the log-rank test. Cox proportional hazards regression models were used to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for survival outcomes. χ^2 tests or Fisher's exact test were applied to categorical variables, such as tumor location or the presence of liver and lung metastases, depending on the expected cell counts. P<0.05 was considered to indicate a statistically significant difference. Subgroup analyses were performed to explore the impact of *RAS* mutation subtypes, metastatic organ involvement, and other clinical factors on survival outcomes. Statistical analyses were conducted using EZR Version 1.63 (Saitama Medical Center, Jichi Medical University, Japan). Results were summarized as means with standard deviations, or medians with interquartile ranges, as appropriate.

Ethical considerations. This study, conducted following The Declaration of Helsinki, was approved by the institutional review board of Osaka International Cancer Institute (approval no. IRB 24122) (24). Given the retrospective nature of our study, which utilized anonymized patient data, the requirement for informed consent was waived.

Results

Patient characteristics and frequencies of RAS mutation subtypes. A total of 796 patients with CRC submitted the MEBGEN RASKET™-B kit at our hospital between August 2018 and December 2023. All samples were available for analysis. Of these, 429 patients with *RAS* wild-type mutations were excluded. The remaining 367 patients with *RAS* mutations included 202 individuals who had received systemic chemotherapy for advanced or recurrent CRC. Among these, 170 patients (84%) had *KRAS* exon 2 mutations, whereas 32 (16%) exhibited minor *RAS* mutations (Fig. 1). Analysis of *RAS* mutation subtypes revealed that *KRAS* codon 12 mutations were the most frequent, accounting for 67% of cases (n=135), followed by *KRAS* codon 13 (17%, n=35) and non-exon 2 mutations (10%, n=20), and *NRAS* mutations (6%, n=12) (Fig. 2). These findings are consistent with those from prior studies, highlighting the predominance of *KRAS* exon 2 mutations in mCRC and the relatively rare occurrence of minor *RAS* mutations (1).

Clinicopathological characteristics of patients with RAS Mutation subtypes. The demographic and clinicopathological characteristics of those patients are shown in Table I. Correlation between *RAS* mutation status and age, sex, ECOG PS, primary site of disease, tumor sidedness, pathological differentiation, microsatellite instability status, previous surgery, previous adjuvant chemotherapy, number of metastatic sites, liver metastasis, lung metastasis was evaluated. Minor *RAS* mutations were more common in left-sided colorectal cancer (81%); however, no significant differences in background were observed between the two groups in any category.

OS in patients with RAS mutation subtypes. The median OS in the *KRAS* exon 2 mutation group was 36.6 months (95% CI: 30.8-38.9), whereas that in the minor *RAS* mutation group was 23.8 months (95% CI: 21.1-not reached) (Fig. 3). The HR for OS between the groups was 0.95 (95% CI: 0.55-1.65, P=0.85), suggesting that the impact of *KRAS* exon 2 mutation and minor *RAS* mutation on prognosis is almost comparable.

OS and PFS in patients receiving first-line bevacizumab-containing therapy. Among 202 patients with *RAS* mutations, 156 received first-line treatment with bevacizumab-containing chemotherapy. The cohort included 134 patients with *KRAS* exon 2 mutations and 22 with minor *RAS* mutations. We investigated the relationship between *RAS* mutation subtype rate and age, sex, ECOG PS, tumor sidedness, number of metastatic sites, liver metastasis, lung metastasis, first-line systemic chemotherapy regimen, and the best efficacy of first-line chemotherapy (Table II). Liver metastases were significantly less common in the minor *RAS* group, reported in only 32% of cases compared to 56% in the *KRAS* exon 2 group (P=0.04). A trend toward improved OS (37.8 vs. 44.9 months, P=0.94) and PFS (11.0 vs. 12.3 months, P=0.46) was not noted between the two groups (Fig. 4).

The efficacy of the first-line chemotherapy was evaluated in 117 patients (100 with *KRAS* exon 2 mutations and 17 with minor *RAS* mutations) with target lesions. Complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) were observed in 1, 48, 39, and 12%, respectively, for the *KRAS* exon 2 mutation group, and 6, 47, 41, and 6%, respectively, for minor *RAS* mutation group (Table III). The overall response rate (ORR) was 49% in patients with *KRAS* exon 2 mutations and 53% in those with minor *RAS* mutations (P=0.80).

Discussion

Minor *RAS* mutations are rare and not fully understood. Therefore, our study provides insights into the distinct clinical and prognostic characteristics of patients with mCRC and minor *RAS* mutations by comparing them with those with *KRAS* exon 2 mutations. Based on our real-world clinical experience, our initial hypothesis posited that minor *RAS* mutations would not confer a worse prognosis than *KRAS* exon 2 mutations.

Table I. Clinical characteristics of patients with *RAS* mutant metastatic colorectal cancer (N=202).

Characteristic	*KRAS* exon 2 mutation (N=170)	Minor *RAS* mutation (N=32)	P-value
Median age, years (range)	66 (26-90)	67 (44-84)	
Sex, female/male	82/88 (48/52)	17/15 (53/47)	0.70
ECOG PS, 0/1/≥2	116/45/9 (68/26/5)	18/11/3 (56/34/9)	0.31
Sidedness, left/right	111/59 (65/35)	26/6 (81/19)	0.10
Differentiation, tub1/tub2/por/sig/muc/pap	65/68/9/0/21/7 (38/40/5/0/12/4)	13/11/3/1/4/0 (41/34/9/3/13/0)	0.30
Microsatellite instability, MSS/MSI-H/unknown	133/1/36 (78/1/21)	28/1/3 (88/3/9)	0.12
Stage IV/recurrence	91/79 (54/46)	14/18 (44/56)	0.34
Previous surgery, yes/no	115/55 (68/32)	18/14 (56/44)	0.23
Previous adjuvant chemotherapy, yes/no	45/125 (26/74)	5/27 (16/84)	0.27
Number of sites of metastasis, 0/1/2/≥3	23/81/50/16 (14/48/29/9)	3/16/11/2 (9/50/34/6)	0.87
Liver metastasis, yes/no	86/84 (51/49)	13/19 (41/59)	0.34
Lung metastasis, yes/no	68/102 (40/60)	15/17 (47/53)	0.56

Data are presented as n (%) unless otherwise specified. Percentages may not total 100 due to rounding. *RAS*, rat sarcoma virus; ECOG, Eastern Cooperative Oncology Group; PS, performance status; tub1/tub2, tubular adenocarcinoma; por, poorly differentiated adenocarcinoma; sig, signet-ring cell carcinoma; muc, mucinous adenocarcinoma; MSS, microsatellite stable; MSI-H, microsatellite instability-high.

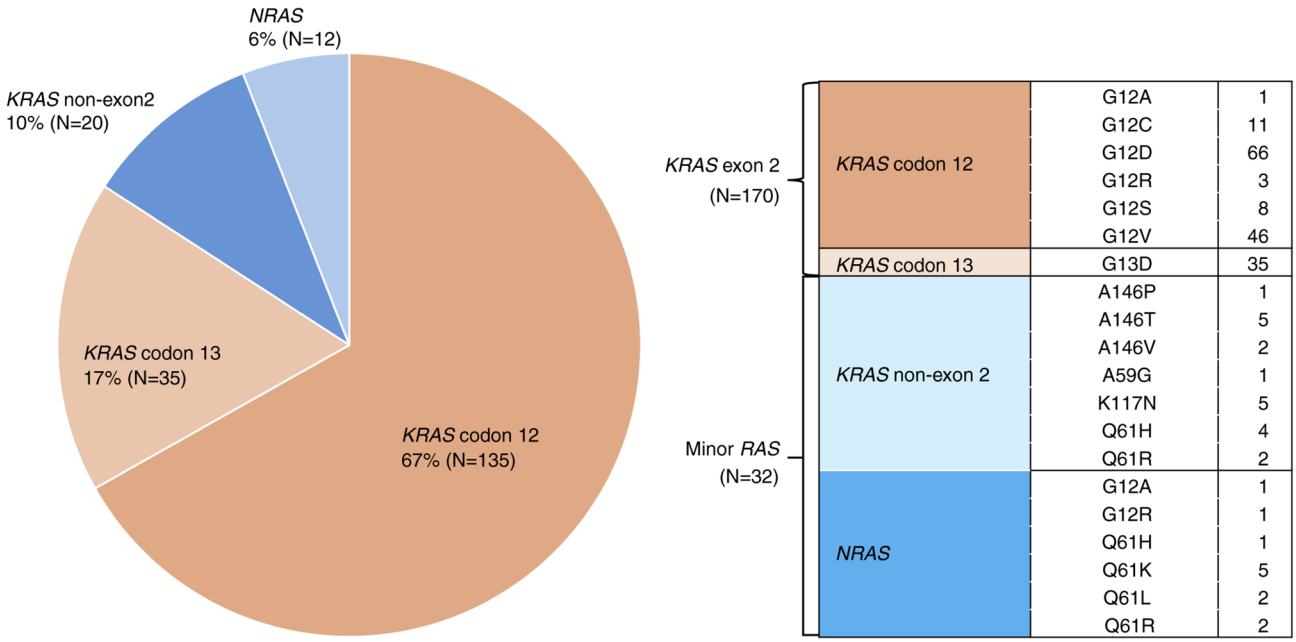

Figure 2. Frequencies of *RAS* mutation subtypes. *RAS*, rat sarcoma virus.

This study investigated the frequency and subtypes of *RAS* mutations (*KRAS* exon 2 and minor *RAS* mutations) and evaluated the prognostic outcomes and the impact of first-line treatment with anti-angiogenic agents in both groups. Our data demonstrated that the median OS and PFS were comparable between the two groups. Consistent with our initial hypothesis, minor *RAS* and *KRAS* exon 2 mutations were suggested to have similar effects on prognosis and treatment outcomes. Accordingly, we believe that current treatment strategies for mCRC with *RAS* mutations remain appropriate and should not be altered based on the *RAS* mutation subtype. While our results align with this view, previous studies have reported conflicting findings. Some have indicated poorer prognoses in patients with minor *RAS* mutations compared to those with *KRAS* exon 2 mutations, whereas others have suggested that *NRAS* mutations may be associated with better outcomes (25-27). Interestingly, despite previous conflicting reports, the relatively favorable survival observed in the minor RAS group may be partially attributable to the lower incidence of liver metastases. Nonetheless, as no current evidence supports a causal relationship between minor RAS mutations and

Table II. Clinical characteristics of patients treated with bevacizumab at first line with *RAS* mutant metastatic colorectal cancer (N=156).

Characteristic	*KRAS* exon2 mutation (N=134)	Minor *RAS* mutation (N=22)	P-value
Median age, years (range)	67 (37-90)	66 (45-79)	
Sex, female/male	65/69 (49/51)	12/10 (55/45)	0.65
ECOG PS, 0/1/≥2	90/38/6 (67/28/4)	15/6/1 (68/27/5)	>0.99
Sidedness, Left/right	82/52 (61/39)	18/4 (82/ 18)	0.09
Number of sites of metastasis, 0/1/2/≥3	5/72/43/14 (4/54/32/10)	2/12/7/1 (9/55/32/5)	0.59
Liver metastasis, yes/no	75/59 (56/44)	7/15 (32/68)	0.04
Lung metastasis, yes/no	58/76 (43/57)	12/10 (55/45)	0.36
First line regimen, triplet/doublet/other	6/108/20 (4/81/15)	1/16/5 (5/73/23)	0.58

Data are presented as n (%) unless otherwise specified. Percentages may not total 100 due to rounding. *RAS*, rat sarcoma virus; ECOG, Eastern Cooperative Oncology Group; PS, performance status.

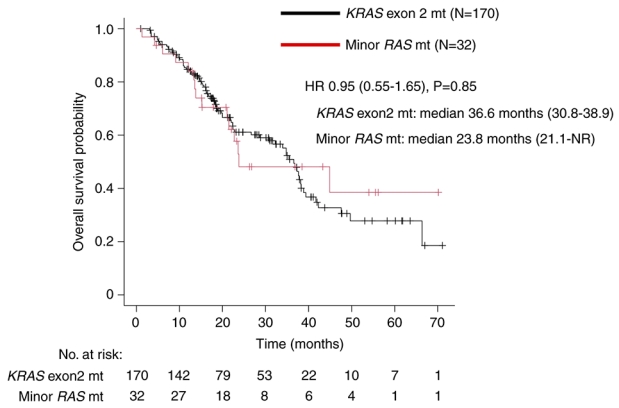

Figure 3. OS in patients with different subtypes of *RAS* mutations. Analysis of OS hazard ratio based on *RAS* mutation status in patients with colorectal cancer using Cox regression analysis (N=202). P-value: Log-rank analysis. OS, overall survival; *RAS*, rat sarcoma virus; HR, hazard ratio; NR, not reached; mt, mutation.

Table III. ORR in patients treated with bevacizumab with different subtypes of *RAS* mutations (N=117).

Response	*KRAS* exon 2 mutation (N=100)	Minor *RAS* mutation (N=17)	P-value
CR	1 (1)	1 (6)	
PR	48 (48)	8 (47)	
SD	39 (39)	7 (41)	
PD	12 (12)	1 (6)	
ORR	49 (49)	9 (53)	0.80

Data are presented as n (%). CR, complete response; ORR, overall response rate; PD, progressive disease; PR, partial response; SD, stable disease.

reduced liver involvement, further studies are warranted to elucidate the biological basis of this observation. In addition to metastatic patterns, molecular differences may also contribute to prognostic variability. For example, Takane *et al* demonstrated that CRC with *NRAS* mutations is associated with a distinct DNA methylation epigenotype (LME) compared to CRC with *KRAS* mutations (26), which could partly explain the differences in prognosis. Furthermore, Ogura *et al* reported that *NRAS* mutations were more prevalent in distal colon cancers compared to *KRAS* mutations, potentially contributing to a more favorable prognosis (27). These conflicting findings highlight the need for larger studies that can analyze *NRAS* and *KRAS* non-exon 2 mutations separately. Moreover, the small number of patients with *NRAS* or *KRAS* non-exon 2 mutations in most studies may limit statistical power and contribute to the inconsistent findings. This study did not assess the pathogenicity of individual minor RAS mutations, and such analysis was beyond the scope of this retrospective investigation.

KRAS mutations were initially recognized solely as biomarkers for resistance to anti-EGFR antibody therapies. However, recent advancements have led to the FDA approval of targeted therapies such as adagrasib plus cetuximab and sotorasib plus panitumumab for *KRAS* G12C mutations, expanding treatment options (28,29). Additionally, new drugs targeting *KRAS* G12D and *KRAS* G12V mutations are currently under development, with promising potential for clinical application. Moreover, several pan-*RAS* inhibitors are being developed, which may eventually address minor *RAS* mutations (30). Since minor *RAS* mutations are rare driver mutations, conducting randomized controlled trials in CRC is challenging. Therefore, the data from our study on the efficacy and survival outcomes of conventional chemotherapy in patients with minor *RAS* mutations could serve as valuable historical control data for future therapeutic developments. These findings may provide a foundational dataset for evaluating the efficacy of novel treatment strategies.

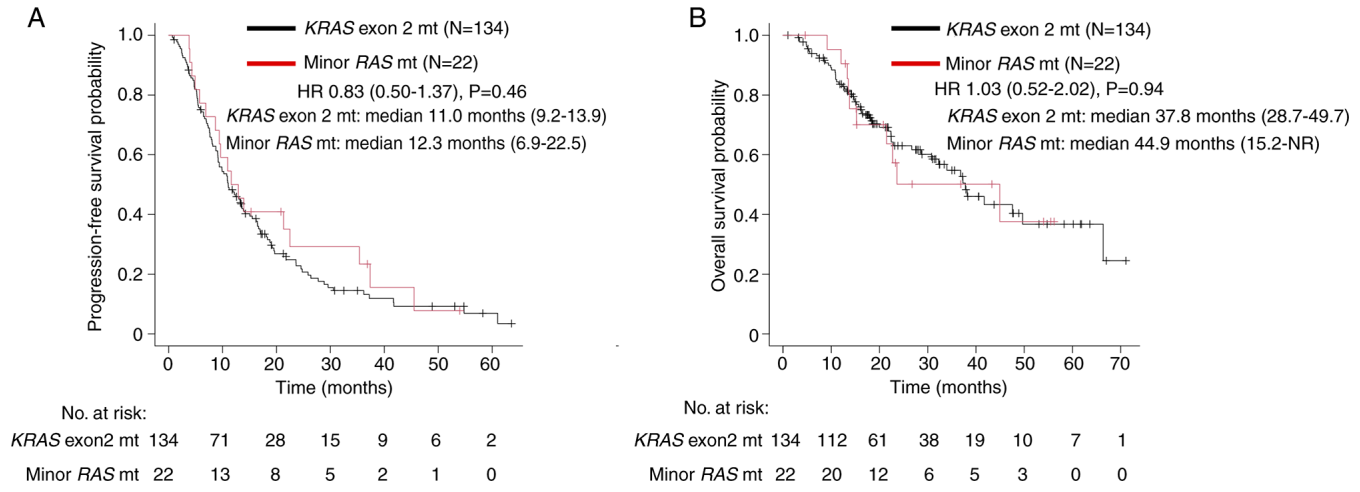

Figure 4. (A) PFS and (B) OS in patients treated with bevacizumab with different subtypes of *RAS* mutations. Analysis of hazard ratios of OS and PFS based on *RAS* mutation status in patients with colorectal cancer using Cox regression analysis (N=156). P-value: Log-rank analysis. PFS, progression-free survival; OS, overall survival; *RAS*, rat sarcoma virus; HR, hazard ratio; NR, not reached; mt, mutation.

We also evaluated the clinical characteristics of patients in both groups. During the study period, the MEBGEN RASKET™-B kit was used to analyze 796 cases of CRC, of which 429 were tumors with wild-type *RAS*. Approximately half of the cases had *RAS* mutations, consistent with previous reports. Among these, *KRAS* exon 2 mutations accounted for 84% (67% *KRAS* codon 12 mutations and 17% *KRAS* codon 13 mutations), whereas minor *RAS* mutations accounted for 16% (10% *KRAS* non-exon 2 mutations and 6% *NRAS* mutations). *KRAS* exon 2 mutations are reportedly present in approximately 35-40% of CRC cases (30-35% *KRAS* codon 12 mutations and 4-8% *KRAS* codon 13 mutations), whereas minor *RAS* mutations are observed in about 10-15% (3-6% of mutations in *KRAS* exons 3 and 4, *NRAS* exons 2 and 3, and less than 1% in *NRAS* exon 4) (17,31). Considering that approximately half of CRC cases harbor *RAS* mutations, our findings regarding the proportion of each *RAS* mutation type within the *RAS*-mutant group were generally consistent with previously reported findings.

An investigation into the distribution of clinicopathological characteristics and *RAS* mutation subtypes revealed no statistically significant findings; however, the proportion of left-sided lesions in minor *RAS* mutations was 81%, indicating a high tendency. Consistent with prior studies, our study identified *NRAS* mutations most frequently in rectal and sigmoid colon cancers (18). Reports have suggested that CIMP-high is associated with a continuous increase in frequency from the rectum to the ascending colon. Conversely, *KRAS* codon 61 and 146 mutations had higher frequencies in cecal cancers, with a higher prevalence of CpG island methylator phenotype (CIMP)-low, compared to *KRAS* wild-type cases (32,33). These discrepancies may be due to small sample sizes in various studies. Notably, a few Japanese studies have reported a higher frequency of *KRAS* non-exon 2 mutations in left-sided CRC that aligns with our findings (25). Despite this predominance, no associations were identified with microsatellite instability (MSI)-H status or pathological differentiation. The biological mechanisms underlying the correlation between minor RAS mutations and left-sided tumors need to be further explored.

As a single-center study with an adequate number of cases, this research represents a significant contribution to understanding the characteristics of minor *RAS* mutations. Future studies with larger sample sizes, combined with data from the recently introduced OncoGuide™ EpiLight™ methylation detection kit in Japan, may help establish the statistical significance of the continuous model for mutation distribution (34-36).

In conclusion, this study elucidates the prognostic impact, clinical characteristics, and effects of anti-angiogenic therapy in patients with minor *RAS* and *KRAS* exon 2 mutations. Consistent with previously reported findings from Japan, minor *RAS* mutations were more common in left-sided colorectal cancer. The association of minor *RAS* mutations with left-sided tumors warrants further investigation into underlying biological mechanisms. The prognostic impact of minor *RAS* mutations appeared to be comparable to that of *KRAS* exon 2 mutations, suggesting that current treatment strategies may remain unchanged for RAS-mutant CRC. However, further studies should validate these findings. The results highlight the necessity of therapeutic advancements targeting all *RAS* mutations to improve the prognosis of mCRC with *RAS* mutations.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

TK and YK conceived and designed the study. YK performed data acquisition. TK and YK confirm the authenticity of all

the raw data. Data analysis was performed by TK and YK. All authors including DS, TO, YA, RM, MK, TS, TO, MN, MI, YK, JN, NS, TY, MT and MY contributed to the interpretation of clinical data. Statistical analysis was conducted by YK. The manuscript was prepared and edited by TK, MT and YK. All authors reviewed the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study, conducted following The Declaration of Helsinki, was approved by the institutional review board of Osaka International Cancer Institute (approval no. IRB 24122). Given the retrospective nature of our study, which utilized anonymized patient data, the requirement for informed consent was waived.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, AI tools (ChatGPT) were used to improve the readability and language of the manuscript, and subsequently, the authors revised and edited the content produced by the AI tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

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