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RESEARCH ARTICLE

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The *RAS* mutation status predicts survival in patients undergoing hepatic resection for colorectal liver metastases: The results from a genetic analysis of all-*RAS*

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Introduction: We investigated the impact of mutations in *KRAS* exons 3-4 and *NRAS* exons 2-3 in addition to *KRAS* exon 2, so-called all-*RAS* mutations, in patients with colorectal liver metastasis (CLM) undergoing hepatic resection.

Methods: We analyzed 421 samples from CLM patients for their all-*RAS* mutation status to compare the overall survival rate (OS), recurrence-free survival rate (RFS), and the pattern of recurrence between the patients with and without *RAS* mutations.

Results: *RAS* mutations were detected in 191 (43.8%). Thirty-two rare mutations (12.2%) were detected in 262 patients with *KRAS* exon 2 wild-type. After excluding 79 patients who received anti-EGFR antibody therapy, 168 were classified as all-*RAS* wild-type, and 174 as *RAS* mutant-type. A multivariate analysis of factors associated with OS and RFS identified the *RAS* status as an independent factor (OS; hazard ratio [HR] = 1.672, $P = 0.0031$, RFS; HR = 1.703, $P = 0.0024$). Recurrence with lung metastasis was observed significantly more frequent in patients with *RAS* mutations than in patients with *RAS* wild-type ($P = 0.0005$).

Conclusions: Approximately half of CLM patients may have a *RAS* mutation. CLM patients with *RAS* mutations had a significantly worse survival rate in comparison to patients with *RAS* wild-type, regardless of the administration of anti-EGFR antibody therapy.

KEYWORDS
all-*RAS* mutation analysis, colorectal liver metastasis, lung-specific recurrence free survival rate

1 | INTRODUCTION

The liver is one of the major sites of distant metastasis from colorectal cancer (CRC).[1] Advances in surgical techniques and chemotherapy have improved the prognosis of the patients with colorectal liver metastasis (CLM) and may lead to better outcomes in CRC patients. Various clinicopathological factors have been identified as predictors of the prognosis of CLM patients.[1,2] However, most of these factors have been evaluated based on the pathological analysis of surgical specimens after hepatic resection.

KRAS mutations are widely known as a predictive biomarker of resistance to anti-epidermal growth factor receptor (EGFR) antibody therapy. Clinical trials have demonstrated that anti-EGFR antibody therapies were largely ineffective in patients with metastatic CRC whose tumors harbored mutations in *KRAS* exon 2.[3–5] Recent studies suggest that, in addition to *KRAS* exon 2, other rare mutations,

including *KRAS* exons 3-4 or *NRAS* exons 2-3, which represent the so called all-*RAS* mutations, confer resistance to anti-EGFR antibody therapy.[6–9] EGFR is one of the most important molecular targets in CRC patients, because the expression of EGFR was considered approximately 60-80% of CRC patients.[10]

Since the 1990s, several studies on the role of *KRAS* mutations as a prognostic factor in CRC patients have been reported.[11–13] However, the results remain controversial. While some studies showed no significant difference in the prognosis between the patients with and without *KRAS* mutations,[11] many reports have suggested that *KRAS* mutations might induce a poorer outcome in terms of recurrence-free survival (RFS), overall survival (OS) and the rate of recurrence especially in the CRC patients with an advanced stage (such as Dukes' C).[12] In our previous study, using a total of 1304 consecutive samples of stage 0-IV CRC to analyze all-*RAS* mutations, a multivariate analysis indicated that *KRAS* and *BRAF* mutations were associated with a poor prognosis in comparison to patients with wild-type.[14] Most of these studies examined the role of the *KRAS* mutations in the prognosis of CRC patients before introduction of anti-EGFR antibody therapy.

Recently, *KRAS* mutations are also recognized as a predictive marker of the prognosis of CLM patients undergoing hepatic resection and their patterns of recurrence.[15–19] The patients with *KRAS* mutation showed poorer outcomes in comparison to the patients with *KRAS* wild type. However, these results were mainly obtained from the analysis of *KRAS* exon 2 alone, not from all-*RAS* analysis.[15–19] Even if *NRAS* and *BRAF* mutations were analyzed in addition to *KRAS* exon 2, these results were based on only a small number of patients (34 patients with *RAS* mutations; 27 *KRAS* and 7 *NRAS*),[20] or a short observation period (median follow-up time <40 months).[20,21] Furthermore, some reports included the patients who received anti-EGFR antibody therapy.[15,16,22]

All-*RAS* mutations are deemed to play two important roles in the clinical course of CLM patients. One of the role is to decrease the effect of the anti-EGFR antibody therapy,[3–9,23] and another role is to have potentials to induce a poor prognosis in CLM patients by uncontrollable tumor progression.[24] Unlike other clinical parameters, the all-*RAS* mutation status can be determined before surgery, using samples from endoscopic biopsy of the primary CRC tumor.[25]

The present study analyzed 421 samples of CLM patients undergoing hepatic resection to investigate the impact of all-*RAS* mutations and to compare prognostic difference between the patients with and without *RAS* mutations, regardless of the effect of the anti-EGFR antibody therapy. We used the clinical data obtained from a long-term observation period (average, >50 months) and evaluated the influence of the all-*RAS* mutation status on the clinical course of CLM patients.

2 | PATIENTS AND METHODS

2.1 | Patients and tissue samples

Between April 1987 and December 2016, 580 patients with CLM underwent hepatic resection at the Saitama Cancer Center. Samples from 456 patients were subjected to a *RAS* mutation analysis. After 35 patients with analysis for *KRAS* exon 2 alone were excluded, 421 samples were used for all-*RAS* mutation analysis.

Tissue samples were surgically excised from each patient. All of the tumor tissue samples were paired with normal tissue samples. The tissue specimens were immediately stored at −80°C. The present study was approved by the Ethics Committee of the Saitama Cancer Center. If no frozen tissue specimens were available, formalin-fixed paraffin-embedded tissue blocks were collected and DNA was extracted from the surgically excised tissue sections.

2.2 | The mutation analysis of *KRAS* and *NRAS*

The methods of the mutation analysis have been described previously.[14] Briefly, genomic DNA was extracted from each sample by the standard SDS-proteinase K procedure, followed by ethanol precipitation. *KRAS* mutations in exons 2 and 3 were detected by denaturing gradient gel electrophoresis (DGGE).[26,27] High resolution melting (HRM) analysis was used to identify mutations in *NRAS* exons 2 and 3 and in *KRAS* exon 4 using a Rotor-Gene Q (Qiagen, Hilden, Germany).

2.3 | The follow-up and analysis of the clinical course after hepatic resection

The clinical data were obtained from the patients' medical records. All patients were regularly screened for recurrence through the monitoring of their CEA levels, and enhanced computed tomography (CT) every 3 months. When recurrence was diagnosed, the patient was aggressively treated using surgical resection or other treatment modalities, including systemic chemotherapy.

2.4 | Statistical analysis

Possible associations between each mutation and the clinicopathological parameters of CLM were assessed using the chi-squared test or Fisher's exact test for categorical variables and the Mann-Whitney *U* or the log-rank test for continuous variables. OS was calculated from the date of surgery to the date of death by any cause or the last follow-up visit. RFS was calculated from the date of surgery to the date of the diagnosis of recurrence. OS and RFS were estimated according to the Kaplan-Meier method. A Cox proportional hazards analysis was used to estimate the clinicopathological and biomarker-specific survival hazard ratios (HR) and 95% confidence intervals (CI). All *P*-values were calculated from two-sided tests. *P*-values of <0.05 were considered to indicate statistical significance. All statistical analyses were performed using the JMP software program (v.11 SAS Institute Inc., Cary, NC).

3 | RESULTS

3.1 | The frequency of *KRAS* and *NRAS* mutations

No mutation was detected in 230 patients of the 421 patients who underwent an all-*RAS* analysis. After excluding 62 patients who

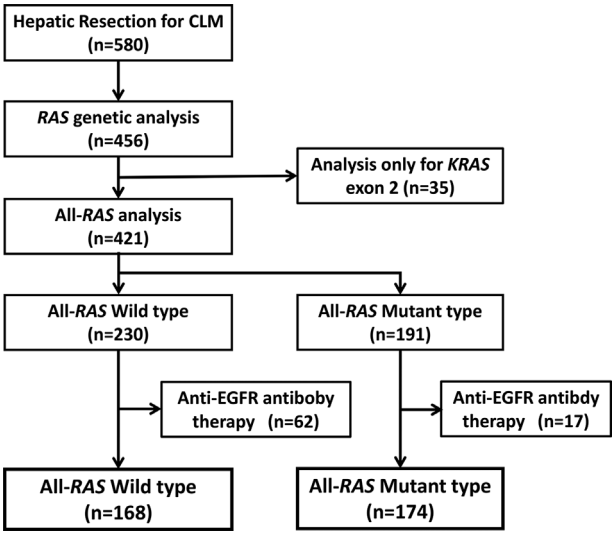

FIGURE 1 Flowchart showing selection of the study population according to all-*RAS* mutation status and excluding the patients who received anti-EGFR antibody therapy. No mutation was detected in 230 patients of the 421 patients who underwent an all-*RAS* analysis. After excluding 62 patients who received anti-EGFR antibody therapy, 168 patients were classified into the all-*RAS* wild-type group. A total of 191 patients (45.4%) had some type of *RAS* mutation. After excluding 17 patients who received anti-EGFR antibody therapy, 174 patients were classified into the all-*RAS* mutant-type group

received anti-EGFR antibody therapy, 168 patients were classified into the all-*RAS* wild-type group (Figure 1, Table 1). *KRAS* exon 2 mutations were detected in 159 patients (37.8%). Among 159 patients with *KRAS* exon 2 mutations, three patients had both mutations in *KRAS* exon 2 and rare mutation, which were in *KRAS* exon 3, *NRAS* exons 2 and 3, respectively. Out of the 262 patients with *KRAS* exon 2 wild-type, mutations of *KRAS* exons 3 and 4, *NRAS* exons 2 and 3 were detected in 11 (4.2%), 9 (3.4%), 6 (2.3%), and 6 (2.3%) patients, respectively. In total, 32 rare mutations (12.2%) were detected and 191 patients (45.4%) had some type of *RAS* mutation. After excluding 17 patients who received anti-EGFR antibody therapy, 174 patients were classified into the all-*RAS* mutant-type group.

3.2 | The impact of the *RAS* mutation status on the clinicopathological characteristics and survival of CLM patients

In the present study, 168 patients and 174 patients were classified into the all-*RAS* wild-type and *RAS* mutant-type groups, respectively. With the exception of two factors, there was no significant difference between the two groups with regard to patient characteristics (Table 2). Tumors of the *RAS* mutant-type group were observed in the proximal colon significantly more frequently than in distal colon or rectum ($P = 0.0004$). Regional lymph-node metastasis of the primary CRC tumor was observed significantly more frequently in *RAS* mutant-type group ($P = 0.0216$).

After a median follow-up period of 52.7 months (range, 1.4-250.9 months), the 5-year OS and 3-year RFS of all 342 patients in both groups were estimated to be 53.6% and 34.3%, respectively. The patients with all-*RAS* mutation had a significantly worse survival rate in comparison to the *RAS* wild-type patients (5-year OS: 42.4% vs 65.3%, $P = 0.0006$, 3-year RFS: 30.0% vs 38.9%, $P = 0.0128$) (Figures 2 and 3). The multivariate analysis to determine the factors associated with OS revealed that age ($P = 0.0394$), the maximum diameter of metastatic liver tumor ($P = 0.0401$), surgical margin ($P = 0.0018$), CEA level before hepatic resection ($P < 0.0001$), regional lymph node metastasis of the primary tumor ($P = 0.0004$), perioperative chemotherapy ($P = 0.0085$), and the *RAS* status (HR = 1.672, 95% CI, 1.188-2.375; $P = 0.0031$) were independent prognostic factors (Table 3). The multivariate analysis to determine the factors associated with RFS also revealed that the *RAS* status (HR = 1.703, 95% CI, 1.206-2.422; $P = 0.0024$) was independent prognostic factor (Table 4).

3.3 | Patterns of recurrence

During the study period, cancer recurrence was diagnosed in 98 *RAS* wild-type patients (58.3%) and 119 *RAS* mutant-type patients (68.4%); the rates of resection of the recurrent tumor were 53.1% and 47.1%, respectively, which did not amount to a significant difference ($P = 0.3787$). The initial recurrence rate with lung metastasis in the *RAS* mutant-type patients ($n = 52$) was significantly higher than that in the *RAS* wild-type patients ($n = 24$) ($P = 0.0005$) (Table 5). The initial

TABLE 1 The frequency of all-*RAS* mutations, including *KRAS* and *NRAS* mutations

	Number of rare mutations						
All-*RAS* mutation analysis ($n = 421$)	*KRAS* exon 2	*KRAS* exon 3	*KRAS* exon 4	*NRAS* exon 2	*NRAS* exon 3	*NRAS* exon 4	Total number of rare mutations in *KRAS* exon 2 wild-type
KRAS exon 2 mutant-type	159 (37.8%)	1	0	1	1	0	
KRAS exon 2 wild-type	262 (62.2%)	11/262 (4.2%)	9/262 (3.4%)	6/262 (2.3%)	6/262 (2.3%)	0	32/262 (12.2%)
All-*RAS* mutant-type	191 (159 + 32) (45.4%)						
All-*RAS* wild-type	230 (54.6%)						

TABLE 2 Patient characteristics of CLM patients with regard to clinicopathological characteristics including all-*RAS* mutation status

Liver metastasis	Total (*n* = 342)	*n*	All-*RAS* wild-type (*n* = 168)	All-*RAS* mutant-type (*n* = 174)	*P*-value (Fisher)	Odds ratio (95%CI)
Gender	M	213	112	101	0.0997	0.692
	F	129	56	73		(0.446-1.074)
Age	≦75 yo	302	121	136	0.3717	1.353
	≧76 yo	40	10	23		(0.695-2.634)
Number of CLMs	≦4	259	130	129	0.4841	1.193
	≧5	83	38	45		(0.727-1.959)
Maximum diameter	≦5 cm	273	131	142	0.4025	0.798
	>5 cm	69	37	32		(0.470-1.355)
Surgical margin	(−)	286	141	145	0.8818	1.044
	(+)	56	27	29		(0.589-1.853)
CEA before	≦5 ng/mL	90	43	47	0.8169	0.945
Hepatic resection	>5 ng/mL	250	123	127		(0.583-1.530)
Primary tumor	Colon	218	105	113	0.6386	0.890
	Rectum	124	63	61		(0.579-1.398)
	Proximal colon	106	37	69	0.0004	1.801
	Distal + Rectum	236	131	105		(1.284-2.526)
Lymph-node meta. of primary tumor	(−)	114	66	48	0.0216	1.699
	(+)	228	102	126		(1.078-2.675)
Perioperative	(+)	150	73	77	0.8814	1.033
Chemotherapy	(−)	192	95	97		(0.674-1.584)
Metachronous		124	65	59	0.3577	1.230
Synchronous		218	103	115		(0.791-1.913)
Dissemination	(−)	301	150	151	0.4754	1.269
Para-aortic LN meta	(+)	41	18	23		(0.658-2.448)

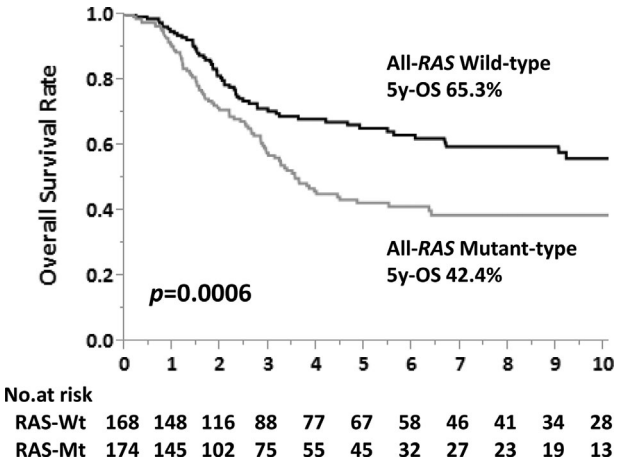

FIGURE 2 Overall survival rate (OS) after hepatic resection by all-*RAS* mutation status. All-*RAS* wild-type group: Black line (▬). All-*RAS* mutant type group: Gray Line (▬). The patients with all-*RAS* mutation had a significantly worse survival rate in comparison to the *RAS* wild-type patients (5-year OS: 42.4% vs 65.3%, *P* = 0.0006)

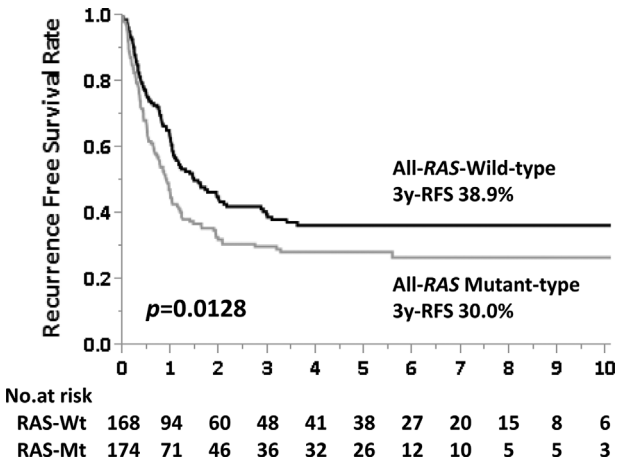

FIGURE 3 Recurrence free survival (RFS) after hepatic resection by all-*RAS* mutation status. All-*RAS* wild type group: Black line (▬). All-*RAS* mutant type group: Gray Line (▬). The patients with all-*RAS* mutation had a significantly worse survival rate in comparison to the *RAS* wild-type patients (3-year RFS: 30.0% vs 38.9%, *P* = 0.0128)

TABLE 3 Univariate and multivariate analysis of the covariates associated with overall survival rate (OS)

Liver metastasis	Total (*n* = 342)	*n*	5-y OS (%)	Univariate, *P*-value	HR (95%CI)	Multivariate, *P*-value	HR (95%CI)
Gender	Male	213	58.0	0.0248	1.456	0.0991	1.334
	Female	129	46.6		(1.044-2.023)		(0.947-1.872)
Age	≦ 75 yo	302	56.3	0.0095	1.795	0.0394	1.657
	≧ 76 yo	40	34.4		(1.119-2.755)		(1.026-2.652)
Number of CLMs	≦ 4	259	58.4	0.0010	1.805	0.2499	1.317
	≧ 5	83	37.9		(1.253-2.559)		(0.821-2.076)
Maximum diameter	≦ 5 cm	273	57.3	0.0005	1.902	0.0401	1.527
	>5 cm	69	39.0		(1.305-2.718)		(1.020-2.245)
Surgical margin	(−)	286	59.5	<0.0001	2.486	0.0018	1.995
	(+)	56	27.5		(1.712-3.544)		(1.299-3.019)
CEA before	≦ 5 ng/mL	90	75.3	<0.0001	2.968	<0.0001	2.779
Hepatic resection	>5 ng/mL	250	45.7		(1.860-5.034)		(1.717-4.769)
Primary tumor	Colon	218	52.6	0.3497	1.180		
	Rectum	124	55.5		(0.839-1.682)		
	Proximal colon	106	48.4	0.1477	1.287		
	Distal + Rectum	236	56.1		(0.908-1.803)		
Lymph-node meta. of primary tumor	(−)	114	69.8	<0.0001	2.223	0.0004	2.041
	(+)	228	46.3		(1.500-3.404)		(1.363-3.161)
Perioperative	(+)	150	60.0	0.0569	1.392	0.0085	1.633
Chemotherapy	(−)	192	48.9		(0.993-1.972)		(1.132-2.381)
Metachronous		124	63.2	1.0143	1.567		
Synchronous		218	48.3		(1.099-2.275)		
All-*RAS*	Wild-type	168	65.3	0.0006	1.792	0.0031	1.672
	Mutant-type	174	42.4		(1.284-2.522)		(1.188-2.375)

TABLE 4 Univariate and multivariate analysis of the covariates associated with recurrence free survival rate (RFS)

Liver metastasis	Total (*n* = 342)	*n*	3-y RFS (%)	Univariate, *P*-value	HR 95%CI	Multivariate, *P*-value	HR 95%CI
Gender	Male	213	38.2	0.1025	1.253		
	Female	129	27.8		(0.952-1.642)		
Age	≦75 yo	302	36.0	0.0059	1.696	0.0465	1.628
	≧76 yo	40	22.0		(1.138-2.444)		(1.008-2.520)
Number of CLMs	≦4	259	39.9	<0.0001	2.098	0.1868	1.372
	≧5	83	16.3		(1.559-2.793)		(0.856-2.168)
Maximum diameter	≦5 cm	273	37.6	0.0001	1.818	0.0398	1.543
	>5 cm	69	21.3		(1.321-2.462)		(1.021-2.295)
Surgical margin	(−)	286	39.3	<0.0001	2.215	0.0014	2.017
	(+)	56	10.9		(1.595-3.021)		(1.317-3.045)
CEA before	≦5 ng/mL	90	58.2	<0.0001	2.264	<0.0001	2.683
Hepatic resection	>5 ng/mL	250	26.2		(1.612-3.264)		(1.659-4.598)
Primary tumor	Colon	218	33.0	0.4940	1.102		
	Rectum	124	36.7		(0.837-1.461)		
	Proximal colon	106	32.3	0.5341	1.095		
	Distal + Rectum	236	35.4		(0.817-1.452)		
Lymph-node meta. of primary tumor	(−)	114	53.8	<0.0001	2.051	0.0002	2.114
	(+)	228	25.3		(1.513-2.829)		(1.403-3.290)
Perioperative	(+)	150	38.0	0.0183	1.384	0.0105	1.612
Chemotherapy	(−)	192	31.2		(1.057-1.820)		(1.117-2.350)
Metachronous		124	47.5	0.0002	1.726	0.8549	1.038
Synchronous		218	27.0		(1.293-2.331)		(0.691-1.542)
All-*RAS*	Wild-type	168	38.9	0.0128	1.402	0.0024	1.703
	Mutant-type	174	30.0		(1.074-1.836)		(1.206-2.422)

TABLE 5 Pattern and resectability of the initial recurrence according to all-*RAS* mutation status

	All-*RAS* wild-type (*n* = 168)	All-*RAS* mutant-type (*n* = 174)	*P*-value (Fisher)	Odds ratio (95%CI)
Recurrence (%)	98 (58.3)	119 (68.4)	0.0533	1.545 (0.992-2.406)
Resectable (%)	52 (53.1)	56 (47.1)	0.3787	0.786 (0.460-1.344)
Liver metastasis	64	72	0.4665	1.175 (0.762-1.811)
Lung metastasis	24	52	0.0005	2.557 (1.490-4.391)
Lymphnode metastasis	12	13	0.9194	1.043 (0.462-2.356)
Peritoneal dissemination	8	23	0.0057	3.027 (1.314-6.976)
Local recurrence	6	6	0.9423	0.958 (0.303-3.028)
Others	4	3	NE	
Brain	0	1		
Bone	4	2		

recurrence rate with peritoneal dissemination in the *RAS* mutant-type patients (*n* = 23) was also significantly higher than that in the *RAS* wild-type patients (*n* = 8) (*P* = 0.0057).

A multivariate analysis of the factors associated with the cumulative incidence of lung-specific RFS (lung-RFS) identified the *RAS* status (HR = 1.959, 95% CI, 1.269-3.077; *P* = 0.0023), number of CLMs (*P* = 0.0025), the CEA level before hepatic resection (*P* = 0.0002), regional lymph-node metastases of the primary tumor (*P* = 0.0197), and perioperative chemotherapy (*P* = 0.0002) were independent factors (Table 6, Figure 4). There was no significant difference between the two groups in the rate of liver-specific RFS (*P* = 0.0638) (Figure 5).

4 | DISCUSSION

The present study investigated the clinical course and prognostic features of 421 CLM patients undergoing hepatic resection, based on the data extracted from previous 1304 CRC patients,[14] and added a genetic analysis of the CLM patients, to investigate the impact of all-*RAS* mutations as a prognostic factor in CLM patients undergoing hepatic resection.

The results of all-*RAS* mutation analyses have been reported in large-scale clinical trials on the effects of the anti-EGFR antibody therapy.[6,23,28] These trials resulted that the additional use of anti-EGFR antibody therapy induced a significant benefit in metastatic CRC patients with all-*RAS* wild-type. However, in patients with *RAS* mutations, the addition of anti-EGFR antibody therapy might induce a poorer outcome in comparison to chemotherapy without anti-EGFR antibody.[6,23]

The frequency of the CRC patients with *KRAS* exon 2 mutations was reported to be 37.0-43.2% (6-7, 11-12, 23, 28). On the other hand, the frequency of the rare mutation was approximately 14.7-26.3% in the patients with *KRAS* exon 2 wild-type and the overall rate of the all-*RAS* mutations in CRC patients was 51.7-55.6%.[6,23,28–30] Since *KRAS* and *NRAS* mutations tend to be mutually exclusive, approximately half of CRC patients may have an *RAS* mutation.

CRC with *KRAS* or *BRAF* mutation were found more frequently in the proximal colon compared with distal colon and rectum, while *NRAS* mutations were found more frequently in the distal colon and rectum.[14,31–32] It was proposed that cellular transformation and mutations occur more frequently in the proximal colon due to close contact of epithelial cells with stimulating bowel content.[32] However, this theory does not explain the higher frequency of *NRAS* mutated tumors in the distal colon and rectum.[14]

Although several studies suggest that anti-EGFR antibody therapy has a negative effect in CRC patients with *NRAS* mutations,[6–8] a multivariate analysis in a previous study revealed that CRC patients with *NRAS* mutations tended to have a favorable prognosis (*P* = 0.059) in comparison to the patients without all-*RAS* mutations.[14] The role of *NRAS* mutations alone remains largely unknown, because the rate of *NRAS* mutations was reported to be 2.2-6.3%[6–8,14,33] and 2.9% (12 of 421 patients) in current study, which was too small to be evaluated.

BRAF is a part of the *RAS/RAF/MAP* signal transduction pathway.[31,34] Patients with *BRAF* mutations showed significantly worse survival in comparison to *KRAS* wild-type patients (*P* = 0.009),[14] and *BRAF* wild-type patients,[31,34] and also showed resistance to anti-EGFR antibody therapy.[6–9] In the current study, 13 CLM patients (3.1%) had a *BRAF* mutation and 11 of 13 (84.6%) had all-*RAS* wild-type. *BRAF* mutations were more frequently detected in CRC patients with all-*RAS* wild-type. We added patients with *BRAF* mutation to the *RAS* mutation group and calculated a prognostic difference by multivariable analysis. Similarly, the *RAS/RAF* mutation group had a poorer prognosis in comparison to *RAS/RAF* wild-type group (data are not shown).

Clinical trials have already demonstrated that anti-EGFR antibody therapies were largely ineffective in CRC patients with all-*RAS* mutations.[6,23,28] Furthermore, in the current study, the CLM patients with all-*RAS* mutations had a significantly worse survival rate in comparison to all-*RAS* wild-type patients, regardless of the administration of anti-EGFR antibody therapy.

Anti-EGFR antibody therapy exhibits antitumor effects by inhibiting multiple EGFR signaling pathways, which are important mechanisms of tumor progression, invasion, and metastasis.[35] In the

TABLE 6 Univariate and multivariate analysis of the covariates associated with lung specific recurrence free survival rate (lung-RFS)

	Total (*n* = 342)	*n*	3-y lung RFS (%)	Univariate, *P*-value	HR 95%CI	Multivariate, *P*-value	HR 95%CI
Gender	Male	213	66.8	0.4516	1.179		
	Female	129	59.0		(0.761-1.802)		
Age	≦75 yo	302	64.7	0.2449	1.453		
	≧76 yo	40	62.2		(0.729-2.619)		
Number of CLMs	≦4	259	68.7	0.0001	2.343	0.0025	2.501
	≧5	83	47.9		(1.475-3.641)		(1.387-4.439)
Maximum diameter	≦5 cm	273	66.8	0.0093	1.881	0.1973	1.425
	>5 cm	69	52.2		(1.134-3.002)		(0.826-2.384)
Surgical margin	(−)	286	67.8	0.0005	2.350	0.1866	1.461
	(+)	56	41.9		(1.400-3.785)		(0.827-2.498)
CEA before	≦5 ng/mL	90	84.7	<0.0001	3.427	0.0002	2.993
Hepatic resection	>5 ng/mL	250	55.1		(1.901-6.829)		(1.630-6.041)
Primary tumor	Colon	218	63.8	0.6888	1.091		
	Rectum	124	64.8		(0.706-1.665)		
	Proximal colon	106	64.6	0.8372	1.050		
	Distal + Rectum	236	64.2		(0.669-1.699)		
Lymph-node meta. of primary tumor	(−)	114	75.5	0.0212	1.726	0.0197	1.755
	(+)	228	57.2		(1.094-2.815)		(1.091-2.913)
Perioperative	(+)	150	70.5	0.0129	1.731	0.0002	2.434
Chemotherapy	(−)	192	58.7		(1.126-2.712)		(1.514-3.989)
Metachronous		124	71.0	0.0397	1.603	0.9460	1.017
Synchronous		218	60.0		(1.030-2.564)		(0.621-1.693)
All-*RAS*	Wild-type	168	72.5	0.0011	2.031	0.0023	1.959
	Mutant-type	174	55.7		(1.323-3.170)		(1.269-3.077)

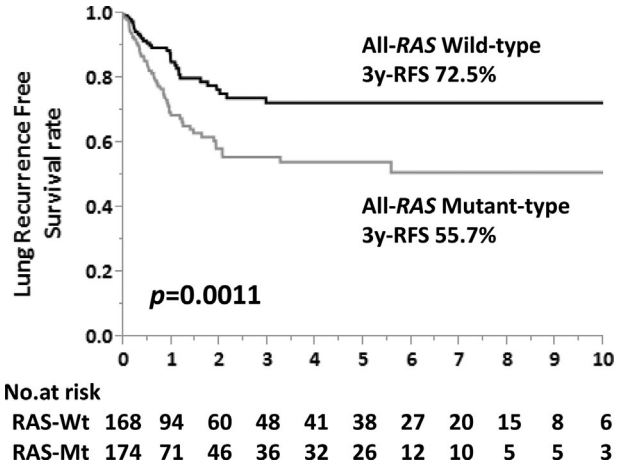

FIGURE 4 Lung-specific recurrence free survival rate (lung-RFS) after hepatic resection by all-*RAS* mutation status. All-*RAS* wild type group: Black line (▬). All-*RAS* mutant type group: Gray Line (▬). The cumulative incidence of lung-RFS in the patients with all-*RAS* mutant type was significantly high in comparison to that in the patients with all-*RAS* wild type ($P = 0.0011$)

presence of anti-EGFR antibodies, signaling is deactivated. In patients with all-*RAS* mutation, uncontrollable tumor progression may be induced through the dysregulation of the oncogenic signaling pathways downstream of EGFR.[24,36]

Anti-vascular endothelial growth factor (VEGF) antibody therapy can be expected to be beneficial for CRC patients with *RAS* mutations.[37,38] In the meta-analysis summarizing the clinical trials comparing the efficacy of anti-EGFR antibody therapy with anti-VEGF antibody therapy in the patients with all *RAS* wild-type, the author suggested addition of anti-EGFR-antibody therapy might be recommended as an initial treatment for metastatic CRC patients with all-*RAS* wild-type, rather than anti-VEGF antibody therapy.[29,30,39,40] However, the optimal additional therapy that achieves the greater clinical benefit remains controversial in all-*RAS* wild-type patients.

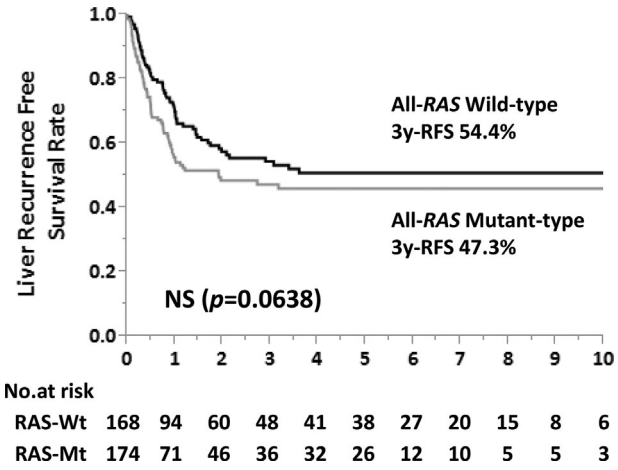

FIGURE 5 Liver-specific recurrence free survival rate (liver-RFS) after hepatic resection by all-*RAS* mutation status. All-*RAS* wild type group: Black line (▬). All-*RAS* mutant type group: Gray Line (▬). There was no significant difference between the two groups in the rate of liver-specific RFS ($P = 0.0638$)

KRAS mutations are recognized as a predictive marker of the prognosis of CLM patients undergoing hepatic resection.[15–19] In CLM patients, *KRAS* mutations were associated with poorer RFS and OS in comparison to the patients with *KRAS* wild type. As for the meta-analysis, one report was based on the analysis only for *KRAS* exon 2 in 12 out of 14 studies, and the other was in 9 out of 11 studies.[15,16] The cumulative incidence of lung recurrence was significantly higher in patients with *KRAS* mutations than patients with *KRAS* wild-type.[18–20] A multivariate analysis indicated that *KRAS* mutations were an independent predictor of lung-RFS. Furthermore, CRC patients with *KRAS* mutations might have a higher rate of initial lung metastasis after the surgical resection of the primary tumors of CRC in comparison to *KRAS* wild-type patients.[41,42] However, these results were mainly obtained from the analysis of *KRAS* exons 2 alone, not from all-*RAS* analysis.[17–19] Similar results were also induced by all-*RAS* analysis.[20–22] Even if these data were obtained by all-*RAS* mutation analysis, these results were based on only a small number of patients (34 patients with *RAS* mutations),[20] or a short observation period (median follow-up time <40 months; 33 and 38 months, respectively).[20,21] These reports evaluated a survival rate with 3-year survival rate[20] or median OS,[21] and did not have enough observation periods to evaluate a 5-year survival rate. One report had enough observation periods (median; 45.6 months) but used the median OS, without evaluating by a 5-year OS[22] In order to eliminate the effect of the anti-EGFR antibody therapy, it is desirable to analyze after excluding the patients who received anti-EGFR antibody therapy. In two meta-analysis, the patients who received the anti-EGFR antibody therapy were included, in 1 of 14 literature and 2 of 11 literature, respectively.[20,21] Also, in other reports using all-*RAS* mutation analysis, the patients who received anti-EGFR antibody therapy were included.[22]

In this study, the initial recurrence rate with lung metastasis after the hepatic resection of CLM patients with all-*RAS* mutations was significantly higher in comparison to CLM patients with all-*RAS* wild-type. The mechanism underlying the high incidence of lung recurrence in patients with all-*RAS* mutations is not clear. However, this pattern of recurrence was one of the most important prognostic factors in CLM patients, and might have been associated with the poor survival rate in the patients with all-*RAS* mutations.[18–20,22]

In the current study, we could confirm that the CLM patients with all-*RAS* mutations had significantly poor survival rate and high incidence of lung recurrence in comparison to all-*RAS* wild-type patients, regardless of anti-EGFR antibody therapy, using clinical data obtained from the all-*RAS* mutation analysis of the 421 CLM patients with long-term observation period (median follow-up >50 months).

It is desirable that all-*RAS* analysis is performed early in the surgical management of CLM patients. Because the all-*RAS* mutation status of the primary tumor and CLM usually showed >95% concordance, endoscopic biopsy, or surgical specimens of primary tumor can be confidently used for the all-*RAS* mutation analysis to predict the

prognosis and for clinical decision-making in relation to anti-EGFR antibody therapy before hepatic resection in CLM patients.[25,43–45]

5 | CONCLUSION

Approximately half of CLM patients may have an *RAS* mutation, which is associated with a poor prognosis even after hepatic resection, regardless of the administration of anti-EGFR antibody therapy. A high incidence of lung recurrence in patients with all-*RAS* mutations might be associated with poor survival. Early in the surgical management of CLM patients, it is important to establish a treatment strategy including perioperative chemotherapy and postoperative surveillance, especially for patients with all-*RAS* mutations.

CONFLICTS OF INTEREST

The authors have no financial conflicts of interest to disclose concerning the presentation using clinical data obtained from the all-*RAS* mutation analysis of the 421 CLM patients

AUTHORS' CONTRIBUTIONS

Katsumi Amikura participated in study concept design acquisition of data analysis interpretation of data the sequence alignment drafted the manuscript revising it critically for important intellectual content. Kiwamu Akagi and Toshiro Ogura carried out the molecular genetic analysis, participated in the acquisition, analysis, or interpretation of the data of genetic analysis, and critical revision. Amane Takahashi and Hirohiko Sakamoto participated in the acquisition of clinical data and critical revision. All authors read and approved the final version of manuscript to be published. And also all authors had agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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