KRAS mutation in patients with metastatic colorectal cancer does not preclude benefit from oxaliplatin- or irinotecan-based treatment

HIROSHI SOEDA[1], HIDEKI SHIMODAIRA[1], MIKA WATANABE[2], TAKAO SUZUKI[3], MAKIO GAMO[4], MASANOBU TAKAHASHI[1], KEIGO KOMINE[1], SHUNSUKE KATO[1] and CHIKASHI ISHIOKA[1]

[1]Department of Clinical Oncology, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Miyagi 980-8575; [2]Department of Pathology, Tohoku University Hospital, Sendai, Miyagi 980-8574; [3]Department of Medical Oncology, Sendai Medical Center, Sendai, Miyagi 983-8520; [4]Department of Clinical Oncology, South Miyagi Medical Center, Ogawara, Miyagi 989-1253, Japan

Received 30 April, 2013; Accepted 10 December, 2013

DOI: 10.3892/mco.2014.254

Abstract. Fluoropyrimidine-based chemotherapy plus antibody therapy is currently the standard first-line treatment for metastatic colorectal cancer (mCRC). In this study, we investigated the hypothesis that mutations in several of the targeted oncogenes are correlated with treatment outcomes in mCRC patients receiving different first-line regimens. Our study included a total of 194 patients who had undergone various forms of first-line chemotherapy. The *KRAS*, *BRAF*, *PIK3CA*, *NRAS* and *AKT1* mutational status of the tumors was assessed and the association between mutational status and treatment outcome was evaluated. The median progression-free survival (mPFS) of the wild-type and mutated *KRAS* subgroups that had received oxaliplatin-based treatment was 8.6 and 6.8 months, respectively (P=0.41), whereas the mPFS of the wild-type *KRAS*, *BRAF*, *PIK3CA*, *NRAS* and *AKT1* subgroups and that of their respective mutant subgroups was 9.7 and 7.2 months, respectively (P=0.10). The mPFS of the wild-type and mutated *KRAS* subgroups that had received irinotecan-based treatments was 7.7 and 9.7 months, respectively (P=0.43). The mPFS of the wild-type *KRAS*, *BRAF*, *PIK3CA*, *NRAS* and *AKT1* subgroups and that of their respective mutant subgroups was 7.1 and 10.0 months, respectively (P=0.76). Our data indicated that mCRC patients with activation of *KRAS*, *BRAF*, *PIK3CA*, *NRAS* and *AKT1* mutations, even those being treated with oxaliplatin- and irinotecan-based regimens as first-line treatment, may benefit from cytotoxic drug therapy.

Correspondence to: Professor Chikashi Ishioka, Department of Clinical Oncology, Institute of Development, Aging and Cancer, Tohoku University, 4-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8575, Japan
E-mail: chikashi@idac.tohoku.ac.jp

Key words: colorectal cancer, *KRAS*, FOLFOX, FOLFIRI

Introduction

Fluoropyrimidine-based chemotherapy plus antibody therapy is currently the standard first-line treatment for metastatic colorectal cancer (mCRC). Infusional 5-fluorouracil (5-FU) and leucovorin (LV) or capecitabine with either oxaliplatin (FOLFOX/XELOX) or irinotecan (FOLFIRI), both of which were shown to have manageable toxicity profiles and to be able to improve treatment efficacy (1-3), are administered as the chemotherapy backbones in such treatment. Due to their proven anticancer activity, the monoclonal antibodies (mAbs) cetuximab, panitumumab and bevacizumab have also been approved for use as first-line chemotherapy in mCRC patients in combination with FOLFOX/XELOX and/or FOLFIRI.

Progress has been achieved with drugs targeting the vascular endothelial growth factor (3) or the epidermal growth factor receptor (EGFR) (4). The EGFR, a receptor tyrosine kinase, triggers a downstream signaling cascade through mechanisms such as the RAS/RAF/MAPK and PI3K/AKT pathways, which are involved in cell proliferation, survival and motility. Based on our knowledge of this cascade, the administration of cetuximab and panitumumab, two mAbs targeting EGFR, was established as a novel treatment option for mCRC patients.

Among the predictive biomarkers used to identify the mCRC patients most likely to benefit from cetuximab and panitumumab treatment, the best established is the *KRAS* gene. Mutations in *KRAS* produce a constitutively active RAS protein, leading in turn to EGFR-independent activation of the RAS/RAF/MAPK pathway (5). The identification of this phenomenon has led to the compelling hypothesis that the activation of *KRAS* mutations may preclude response to anti-EGFR mAb therapy, a hypothesis supported by earlier clinical observations (6,7). Between 2007 and 2008, 6 randomized clinical trials were conducted, in which the *KRAS* status was retrospectively assessed in tumor samples from mCRC patients who had been randomly assigned to receive panitumumab (8,9) or cetuximab treatment (10-13).

The activation of mutations of *BRAF*, another component of the EGFR/MAPK signal transduction pathway, is also prevalent

among mCRC patients. As with the *KRAS* mutation, it is plausible that *BRAF* mutations may confer resistance to anti-EGFR therapy, although their lower prevalence makes this hypothesis more difficult to test clinically. However, two previous studies reported that *BRAF* mutation at codon 600 (V600E), resulting in strong activation of the BRAF protein downstream of *KRAS*, is associated with a shorter progression-free survival (PFS) and overall survival in mCRC chemorefractory patients treated with anti-EGFR mAb therapy (14,15).

Tumor-derived mutant *PI3K* was shown to stimulate the AKT pathway and promote cell growth in several types of cancer, including CRC. Tumors with *PIK3CA* mutations have been associated with poor prognosis, with mutations in the *PIK3CA* gene found to significantly impair the response to anti-EGFR mAb treatment in mCRC patients. In support of these findings, a large-scale European study reported that acquiring knowledge regarding the combined *KRAS*, *BRAF*, *PIK3CA* and *NRAS* mutation status may improve the sensitivity of prediction of the response to anti-EGFR mAb therapy (16).

In order to counsel mCRC patients harbouring *KRAS* mutations (and possibly other gene mutations), we need to establish whether they may still benefit from standard chemotherapeutic options. Therefore, this study pursued three objectives: to evaluate the efficacy of the currently available chemotherapeutic protocols for the treatment of mCRC patients, to investigate the value of predictive biomarkers in the personalization of 5-FU-based chemotherapy and to test the hypothesis that mutations in several of the targeted oncogenes are correlated with treatment outcomes in patients receiving different first-line regimens. To test this hypothesis, we first evaluated the predictive significance of *KRAS*, *BRAF*, *PIK3CA*, *NRAS* and *AKT1* mutations in a cohort of mCRC patients who had undergone 5-FU-based chemotherapy; subsequently, using a uniform catalog of retrospective but detailed clinical data, we determined the predictive value of these mutations regarding patient outcomes following completion of the most common therapeutic regimens. This analysis allowed for assessment of the predictive significance of *KRAS*, *BRAF*, *PIK3CA*, *NRAS* and *AKT1* mutations independent of anti-EGFR therapy and their predictive value regarding benefit from oxaliplatin- or irinotecan-based therapy.

Materials and methods

Patients and treatment methods. This study was approved by the Ethics Committee of Tohoku University School of Medicine and included a total of 194 mCRC patients who had received various forms of first-line chemotherapy at the study site between February, 2005 and October, 2010.

The mFOLFOX6 regimen consisted of a 2-h infusion of 85 mg/m^2 of oxaliplatin on day 1, a 2-h infusion of 200 mg/m^2 of LV on day 1, a bolus of 400 mg/m^2 of 5-FU on day 1 and a 46-h infusion of 2,400 mg/m^2 of 5-FU/day on days 1-2. The FOLFIRI regimen consisted of a 1.5-h infusion of 150 mg/m^2 of irinotecan on day 1, a 2-h infusion of 200 mg/m^2 of LV on day 1, a bolus of 400 mg/m^2 of 5-FU on day 1 and a 46-h infusion of 2,400 mg/m^2 of 5-FU/day on days 1-2. The treatments had been administered on day 1 and repeated on day 2 of a 14-day treatment cycle. The IRIS regimen consisted of continuous administration of 150 mg/m^2 of irinotecan for 90 min on day 1, followed by twice-daily administration of S-1 for a 2-week period on days 3-16. The administered dose of S-1 had been determined as follows: for a body surface area (BSA) of <1.25 m^2, 80 mg/day; for a BSA of 1.25-1.5 m^2, 100 mg/day; and for a BSA of >1.5 m^2, 120 mg/day as a 3-week course.

Table I. First-line treatment regimens used in this retrospective study (n=194).

Regimens	No. (%)
FOLFOX + bevacizumab	27 (13.9)
FOLFOX	82 (42.3)
FOLFIRI + bevacizumab	17 (8.8)
FOLFIRI	26 (13.4)
IRIS + bevacizumab	9 (4.6)
IRIS	13 (6.7)
5-Fluorouracil only	17 (8.8)
No treatment	3 (1.5)
Oxaliplatin-based treatment	109 (56.2)
Irinotecan-based treatment	65 (33.5)

FOLFOX, 5-fluorouracil, leucovorin and oxaliplatin; FOLFIRI, 5-fluorouracil, leucovorin and irinotecan; IRIS, irinotecan and S-1.

Tumor collection and processing. Formalin-fixed, paraffin-embedded (FFPE) samples of tumor tissue from archival specimens that had been collected at the time of diagnosis and stored at Tohoku University Hospital were investigated. The assays of the tissue samples for *KRAS*, *BRAF*, *PIK3CA*, *NRAS* and *AKT1* mutations were performed at the Department of Clinical Oncology, Institute of Development, Aging and Cancer, Tohoku University. All samples were screened for *KRAS* mutations in codons 12, 13 and 61; *BRAF* V600E; *PIK3CA* mutations in exons 9 and 20; *NRAS* mutations in codons 12, 13 and 61; and *AKT1* E17K. All samples were also classified as mutant or wild-type.

Nucleotide sequence analysis. Mutation analyses of *KRAS*, *BRAF*, *PIK3CA*, *NRAS* and *AKT1* were performed by extraction of genomic DNA from FFPE tissue slides or sections. DNA was extracted using the QIAamp DNA FFPE tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Analyses of the DNA sequences were performed using the automated CEQ2000XL DNA analysis system (Beckman Coulter, Fullerton, CA, USA) under specific cycle and temperature conditions. The PCR products were analyzed by 1.0% agarose gel electrophoresis. Appropriate positive and negative controls were included for the *KRAS*, *BRAF*, *PIK3CA*, *NRAS* and *AKT1* analyses. To minimize bias, all researchers who performed the mutation analyses were blinded to the clinical outcomes.

Statistical analysis. All patients for whom data regarding *KRAS*, *BRAF*, *PIK3CA*, *NRAS* and *AKT1* mutation status were available, were included in the analysis. The response rate (RR) was determined according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.0. According to RECIST, the patients were categorized as responders if they

Table II. *KRAS*, *BRAF*, *PIK3CA*, *NRAS* and *AKT1* mutation frequencies (n=194).

Gene	Codon	Nucleotide substitution	Amino acid substitution	No. (%)	Total (%)
KRAS	12	GGT→CGT	G12R	1 (0.5)	78 (40.2)
		GGT→TGT	G12C	3 (1.5)	
		GGT→GAT	G12D	25 (12.9)	
		GGT→GCT	G12A	2 (1.0)	
		GGT→GTT	G12V	20 (10.3)	
	13	GGC→TGC	G13C	1 (0.5)	
		GGC→GAC	G13D	22 (11.3)	
	61	CAA→CGA	Q61R	1 (0.5)	
		CAA→CAC	Q61H	2 (1.0)	
		CAA→CAT	Q61H	1 (0.5)	
BRAF	600	GTG→GAG	V600E	10 (5.2)	10 (5.2)
PIK3CA	542	GAA→AAA	E542K	4 (2.1)	23 (11.9)
	545	GAG→AAG	E545K	4 (2.1)	
		GAG→GGG	E545K	7 (3.6)	
	546	CAG→AAG	Q546K	2 (1.0)	
	1047	CAT→TAT	H1047Y	1 (0.5)	
		CAT→CTT	H1047L	1 (0.5)	
		CAT→CGT	H1047R	4 (2.1)	
NRAS	12	GGT→GAT	G12D	3 (1.5)	3 (1.5)
AKT1	17	GAG→AAG	E17K	2 (1.0)	2 (1.0)
KRAS and *PIK3CA*				9 (4.7)	

achieved complete response (CR) or partial response (PR) and as non-responders if they exhibited stable disease (SD) or progressive disease (PD). The associations between treatment response or patient characteristics and mutational status were assessed using the χ^2 test. PFS was defined as the time interval between the initiation of chemotherapy and the first objective evidence of disease progression or death from any cause. The PFS was determined using the Kaplan-Meier method and compared using the log-rank test. Statistical significance was set at a level of $P<0.05$ for a bilateral test. Through such means, the *KRAS* mutational status was investigated and the hypothesis that PFS varies according to the type of first-line regimen (oxaliplatin- or irinotecan-based) was tested.

Results

Study objective. This retrospective study investigated the efficacy of first-line chemotherapeutic protocols in 194 mCRC patients according to gene status and its association with several patient clinical characteristics. As tumor samples and complete end-point data were available for all patients, RR and PFS were determined for the entire patient sample (100%).

Treatment regimens. Combination chemotherapy was administered as first-line treatment to 174 patients (89.7%), either with or without mAb supplementation (Table I). 5-FU was administered as the only cytotoxic agent to 17 patients (8.8%). A total of 109 patients (56.2%) were treated with oxaliplatin in the first-line setting. The oxaliplatin-containing regimen consisted of only the FOLFOX regimen (infusion and bolus 5-FU plus oxaliplatin). A total of 65 patients (33.5%) were treated with irinotecan in the first-line setting. The irinotecan-containing regimen consisted of the FOLFIRI regimen (infusion and bolus 5-FU with irinotecan) for 43 patients and S-1 plus irinotecan for 22 patients. As first-line treatment, bevacizumab was administered as part of an oxaliplatin-containing regimen to 27 patients (13.9%) and with irinotecan to 26 patients (13.4%). As bevacizumab was not approved until 2007 in Japan, the percentage of mCRC patients who received bevacizumab in this study was low (27.3%).

Mutation analyses of KRAS, BRAF, PIK3CA, NRAS and AKT1. Table II lists the mutations detected by direct sequencing. A relatively rare mutation in codon 61 was analyzed in addition to the common mutations in codons 12 and 13 in order to increase the sensitivity of mutation detection. *KRAS* mutations at codons 12, 13 and 61 were observed in 78 (40.2%) of the tumor samples. Of the 78 detected mutations in codons 12 and 13, the most frequent was G12D (12.9%), followed by G13D (11.3%), G12V (10.3%), G12C (1.5%), G12A (1.0%), G12R (0.5%) and G13C (0.5%). In codon 61, Q61H and Q61R were detected in 4 samples (2.0%). Three common *KRAS* mutations, G12D, G13D and G12V, were also frequently detected. V600E was detected in 10 samples (5.2%), all of which harboured wild-type *KRAS*. *PIK3CA* mutations in exon 9 (E542K, E545K, E545G and Q546K) were detected in 17 samples (8.8%) and

Table III. Patient characteristics.

Characteristics	All	*KRAS* wild-type	*KRAS* mutant	P-value
Total number of patients	194	116	78	
Median age, [years (range)]	63 (16-82)	62 (16-82)	65 (37-81)	
Gender				
Male	111	73	38	0.0498
Female	83	43	40	
Primary tumor				
Cecum	10	4	6	0.70
Ascending colon	42	23	19	
Transverse colon	15	9	6	
Descending colon	10	6	4	
Sigmoid colon	42	25	17	
Rectum	75	49	26	
Metastatic sites				
Liver	114	64	50	0.10
Lung	100	52	48	
Intra-abdominal lymph nodes	72	51	21	
Peritoneum	36	16	20	
Bone	10	5	5	
Others	22	12	10	

Table IV. Response to oxaliplatin-based treatment according to the presence or absence of gene mutations (n=109).

	KRAS status		Genetic status of *KRAS*, *BRAF*, *PIK3CA*, *NRAS* and *AKT1*		
Tumor response	Mutant (%)	Wild-type (%)	Mutant of any genes (%)	Wild-type of all genes (%)	Total (%)
Total	43 (100)	66 (100)	58 (100)	51 (100)	109 (100)
Bevacizumab use	11 (25.6)	16 (24.2)	15 (25.9)	12 (23.5)	27 (24.8)
CR	1 (2.3)	1 (1.5)	1 (1.7)	1 (2.0)	2 (1.8)
PR	20 (46.5)	33 (50.0)	25 (43.1)	28 (54.9)	53 (48.6)
SD	14 (32.6)	25 (37.9)	22 (37.9)	17 (33.3)	39 (35.8)
PD	8 (18.6)	7 (10.6)	10 (17.2)	5 (9.8)	15 (13.8)
RR (%)	48.8	51.5	44.8	56.9	50.5
DCR (%)	81.4	89.4	82.8	90.2	86.2
mPFS (months)	6.8	8.6	7.2	9.7	8.1

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; RR, response rate; DCR, disease control rate; mPFS, median progression-free survival.

PIK3CA mutations in exon 20 (H1047R, H1047L and H1047Y) in 6 (3.1%). Mutations in *KRAS* and *PIK3CA* were detected in 9 samples (4.7%). *NRAS* mutations at codons 12, 13 and 61 were detected in 3 samples (1.5%); and an *AKT1* mutation at codon 17 (E17K) was detected in two samples (1.0%).

Patient characteristics. The characteristics of the 194 mCRC patients (median age, 63 years; range, 16-82 years) from whom primary tumor tissue samples had been collected were retrospectively analyzed (Table III). The most frequent type of tumor was tumor of the rectum (75 patients; 38.7%), followed by tumor of the ascending and sigmoid colon (42 patients; 21.6%), transverse colon (15 patients; 7.7%) and descending colon and cecum (10 patients; 5.2%). The most frequent site of metastasis was the liver (114 patients; 58.8%), followed by the lungs (100 patients; 51.5%), intra-abdominal lymph nodes (72 patients; 37.1%) and the peritoneum (36 patients; 18.6%).

Effect of mutation status on the outcome of first-line chemotherapy. Tables IV and V show the results of the analysis of

Table V. Response to irinotecan-based treatment according to the presence or absence of gene mutations (n=65).

	KRAS status		Genetic status of *KRAS*, *BRAF*, *PIK3CA*, *NRAS* and *AKT1*		
Tumor response	Mutant (%)	Wild-type (%)	Mutant of any genes (%)	Wild-type of all genes (%)	Total (%)
Total	26 (100)	39 (100)	33 (100)	32 (100)	65 (100)
Bevacizumab use	9 (34.6)	17 (43.6)	12 (36.4)	14 (43.8)	26 (40.0)
CR	2 (7.7)	1 (2.6)	2 (6.1)	1 (3.1)	3 (4.6)
PR	12 (46.2)	19 (48.7)	15 (45.5)	16 (50.0)	31 (47.7)
SD	8 (30.8)	14 (35.9)	12 (36.4)	10 (31.3)	22 (33.8)
PD	4 (15.4)	5 (12.8)	4 (12.1)	5 (15.6)	9 (13.8)
RR (%)	53.8	51.3	51.5	53.1	52.3
DCR (%)	84.6	87.2	87.9	84.4	86.2
mPFS (months)	9.7	7.7	10.0	7.1	9.1

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; RR, response rate; DCR, disease control rate; mPFS, median progression-free survival.

the association between clinical response in terms of RR and median PFS (mPFS) and the presence or absence of gene mutations. There were no significant differences in RR or mPFS between the wild-type and mutant *KRAS* subgroups who had received oxaliplatin- or irinotecan-based treatment as first-line therapy. Furthermore, there was no significant difference in RR or mPFS between the wild-type *KRAS*, *BRAF*, *PIK3CA*, *NRAS* and *AKT1* subgroups and the respective mutant subgroups in any of the 5 genes.

The mPFS of the wild-type and mutant *KRAS* subgroups who had received oxaliplatin-based treatment was 8.6 (n=66) and 6.8 months (n=43), respectively (P=0.41; Fig. 1A). Of the 109 assessed patients, 16 of the 66 (24.2%) patients in the wild-type subgroup and 11 of the 43 (25.6%) patients in the mutant *KRAS* subgroup had received bevacizumab in combination with FOLFOX (P=0.87). The mPFS of the wild-type *KRAS*, *BRAF*, *PIK3CA*, *NRAS* and *AKT1* subgroups and that of their respective mutant subgroups was 9.7 (n=51) and 7.2 months (n=58), respectively (P=0.10; Fig. 1B).

The mPFS of the wild-type and mutant *KRAS* subgroups who had received irinotecan-based treatments was 7.7 (n=39) and 9.7 months (n=26), respectively (P=0.43; Fig. 2A). Of the 65 assessed patients, 17 of the 39 (43.6%) patients in the wild-type subgroup and 9 of the 26 (34.6%) patients in the mutant *KRAS* subgroup had received bevacizumab in combination with FOLFIRI or IRIS treatment (P=0.47). The mPFS of the wild-type *KRAS*, *BRAF*, *PIK3CA*, *NRAS* and *AKT1* subgroups and that of their respective mutant subgroups were 7.1 (n=32) and 10.0 months (n=33), respectively (P=0.76; Fig. 2B).

Discussion

This analysis of various mutations of the *KRAS*, *BRAF*, *PIK3CA*, *NRAS* and *AKT1* genes in 194 Japanese mCRC patients resulted in the detection of *KRAS* mutations at a frequency (78/194; 40.2%) similar to that described in a previous study of Japanese patients (17). As the pattern of *KRAS* mutations was also found to be similar to that reported in a previous study on Caucasian patients (18,19), the results indicated that *KRAS* mutations do not differ significantly between Japanese and Caucasian populations in terms of frequency and mutation spectrum. The prevalence of *PIK3CA* mutation (23/194; 11.9%) was also found to be similar to that reported by previous studies (10-20%) (16). By contrast, the prevalence of *BRAF* mutations (10/194; 5.2%) was found to be lower compared to that reported in studies on Caucasian patients (20), possibly reflecting the genetic differences between the populations. Of the mutations detected, E542K, E545K and H1047R were identified as hotspot mutations, whereas the E545G mutation was rarely detected (16,21). A large-scale analysis is required to elucidate whether this discrepancy in the mutation spectrum is the result of genetic differences among different populations. Previous studies identified mutations in the *NRAS* and *AKT1* genes in 2.6% (16) and 5.9% (22) of mCRC patients, respectively. In this study, *NRAS* and *AKT1* mutations were detected in 1.5% (3 patients) and 1.0% (2 patients) of the sample, respectively.

Similar to previous investigations, the present study analyzed the association between gene mutations and patient characteristics in order to determine whether such associations may predict the efficacy of a first-line regimen. Sartore-Bianchi *et al* (23) reported that *KRAS* mutations were significantly more prevalent among females compared with males, whereas *PIK3CA* mutations were not found to be significantly associated with gender. In accordance with Watanabe *et al* (17), who reported a higher prevalence of *KRAS* mutations among Japanese female (40.9%) compared to male mCRC patients (35.5%; P=0.001), a higher prevalence of *KRAS* mutations was detected among the samples obtained from female (48.2%) compared with those obtained from male patients (34.2%, P=0.050) in the present study.

The individualization of drug therapy for mCRC patients is becoming increasingly feasible. Studies on patients receiving first-line and subsequent lines of treatment demonstrated that those with *KRAS* mutations do not respond to or experience any survival benefit from treatment with anti-EGFR mAb

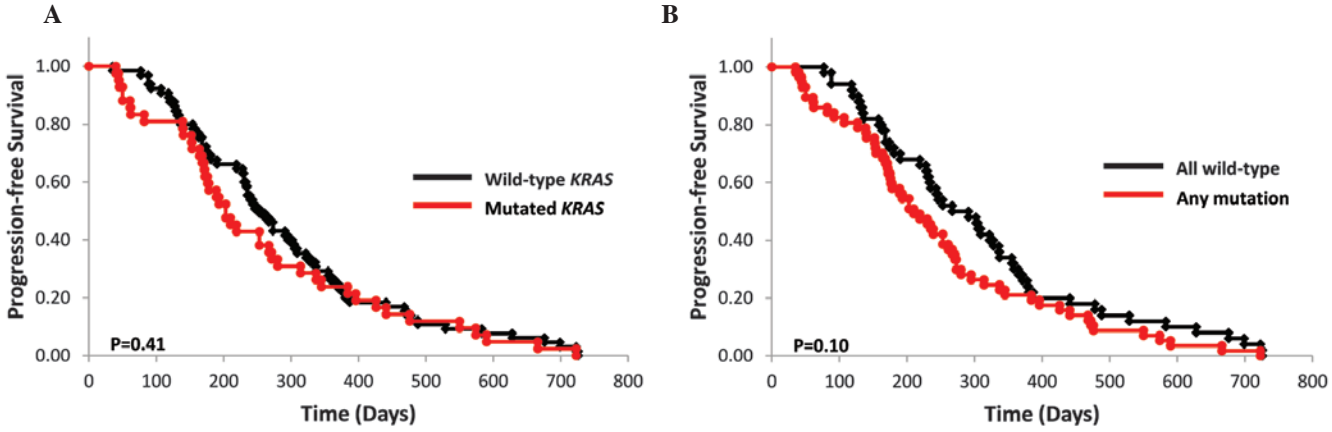

Figure 1. Kaplan-Meier curve of cumulative progression-free survival according to *KRAS*, *BRAF*, *PIK3CA*, *NRAS* and *AKT1* mutational status in metastatic colorectal cancer patients treated with oxaliplatin-based therapy as first-line treatment. (A) Comparison between wild-type and mutant *KRAS* subgroups. (B) Comparison between wild-type and mutant *KRAS*, *BRAF*, *PIK3CA*, *NRAS* and *AKT1* subgroups.

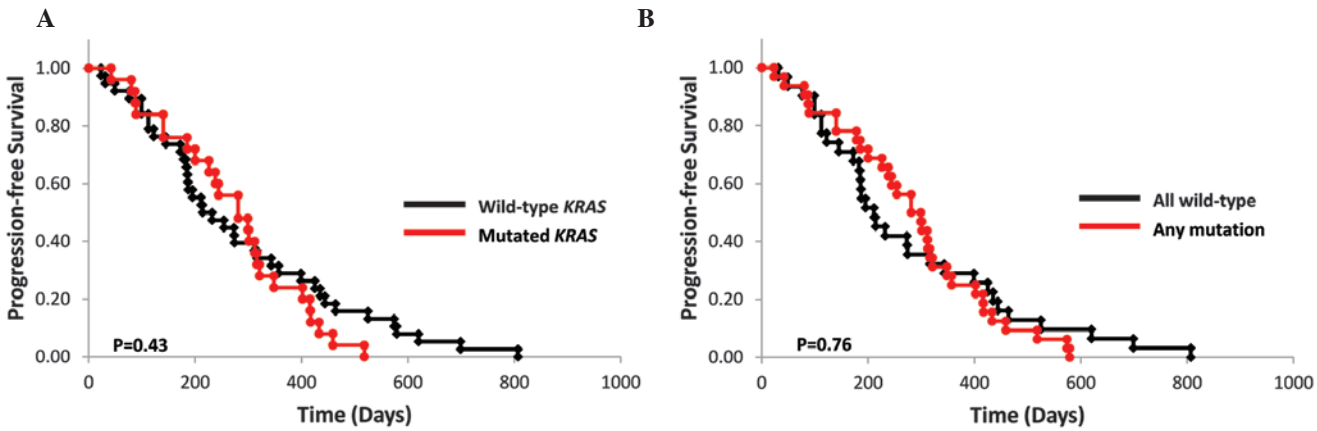

Figure 2. Kaplan-Meier curve of cumulative progression-free survival according to *KRAS*, *BRAF*, *PIK3CA*, *NRAS* and *AKT1* mutational status in metastatic colorectal cancer patients treated with irinotecan-based therapy as first-line treatment. (A) Comparison between wild-type and mutant *KRAS* subgroups. (B) Comparison between wild-type and mutant *KRAS*, *BRAF*, *PIK3CA*, *NRAS* and *AKT1* subgroups.

therapy. Based on this finding, all mCRC patients are currently offered *KRAS* testing to determine whether their tumor is wild-type *KRAS* and, if so, counseled that they would likely benefit from anti-EGFR mAb therapy. As such, it is crucial to establish whether the mutation may affect the ability to benefit from anti-EGFR mAb therapy (or any other form of therapy), or whether prognosis is independent of treatment. Retrospective analyses of *KRAS* mutations in mCRC patients treated with bevacizumab plus chemotherapy revealed that the clinical benefit of bevacizumab is independent of the *KRAS* status (24,25). Other studies investigated the association between *KRAS* status (as well as other gene statuses) and clinical benefit from oxaliplatin- or irinotecan-based treatment in the first-line setting (26,27). Those studies reported that the clinical benefit of oxaliplatin- or irinotecan-based treatment is independent of the *KRAS* mutational status.

In this study, the patients who had received oxaliplatin treatment exhibited longer mPFS in the wild-type *KRAS* alleles. By contrast, the patients who had received irinotecan treatment exhibited longer mPFS in the mutant *KRAS* alleles. Although there were no statistically significant differences in the distinct *KRAS* status between the oxaliplatin- and irinotecan-based treatment groups, the *KRAS* status is likely to affect the outcome of these treatments in some of the patients. The results of the present study indicated that mCRC patients with activation of *KRAS* mutations, even those treated with oxaliplatin- and irinotecan-based regimens as first-line treatments, may benefit from cytotoxic drug therapy. We also provided evidence that both the wild-type and mutant *KRAS* subgroups of mCRC patients may benefit from oxaliplatin- and irinotecan-based therapy.

Acknowledgements

This study was supported in part by grants-in-aid from the Ministry of Education, Science, Sports and Culture of Japan. We would like to thank Eri Yokota for assisting with the mutational analysis, Hiroyoshi Suzuki for preparing the samples at the Sendai Medical Center and Yayoi Takahashi for preparing the samples at the Tohoku University Hospital.

References

1. Van Cutsem E, Kohne CH, Hitre E, *et al*: Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med 360: 1408-1417, 2009.

2. Bokemeyer C, Bondarenko I, Makhson A, *et al*: Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. J Clin Oncol 27: 663-671, 2009.
3. Hurwitz H, Fehrenbacher L, Novotny W, *et al*: Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 350: 2335-2342, 2004.
4. Cunningham D, Humblet Y, Siena S, *et al*: Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 351: 337-345, 2004.
5. Bos JL, Fearon ER, Hamilton SR, *et al*: Prevalence of ras gene mutations in human colorectal cancers. Nature 327: 293-297, 1987.
6. Benvenuti S, Sartore-Bianchi A, Di Nicolantonio F, *et al*: Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. Cancer Res 67: 2643-2648, 2007.
7. Di Fiore F, Blanchard F, Charbonnier F, *et al*: Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by cetuximab plus chemotherapy. Br J Cancer 96: 1166-1169, 2007.
8. Amado RG, Wolf M, Peeters M, *et al*: Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol 26: 1626-1634, 2008.
9. Hecht JR, Mitchell E, Chidiac T, *et al*: A randomized phase IIIB trial of chemotherapy, bevacizumab, and panitumumab compared with chemotherapy and bevacizumab alone for metastatic colorectal cancer. J Clin Oncol 27: 672-680, 2009.
10. Van Cutsem E, Lang I, D'haens G, *et al*: KRAS status and efficacy in the first-line treatment of patients with metastatic colorectal cancer (mCRC) treated with FOLFIRI with or without cetuximab: the CRYSTAL experience. J Clin Oncol 26: abstract 2, 2008.
11. Punt CJ, Tol J, Rodenburg CJ, *et al*: Randomized phase III study of capecitabine, oxaliplatin, and bevacizumab with or without cetuximab in advanced colorectal cancer (ACC), the CAIRO2 study of the Dutch Colorectal Cancer Group (DCCG). J Clin Oncol 26: abstract LBA4011, 2008.
12. Bokemeyer C, Bondarenko I, Hartmann JT, *et al*: KRAS status and efficacy of first-line treatment of patients with metastatic colorectal cancer (mCRC) with FOLFOX with or without cetuximab: the OPUS experience. J Clin Oncol 26: abstract 4000, 2008.
13. Karapetis CS, Khambata-Ford S, Jonker DJ, *et al*: K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med 359: 1757-1765, 2008.
14. Di Nicolantonio F, Martini M, Molinari F, et al: Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. J Clin Oncol 26: 5705-5712, 2008.
15. Tol J, Nagtegaal ID and Punt CJ: BRAF mutation in metastatic colorectal cancer. N Engl J Med 361: 98-99, 2009.
16. De Roock W, Claes B, Bernasconi D, *et al*: Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. Lancet Oncol 11: 753-762, 2010.
17. Watanabe T, Yoshino T, Uetake H, *et al*: KRAS mutational status in Japanese patients with colorectal cancer: results from a nationwide, multicenter, cross-sectional study. Jpn J Clin Oncol 43(7): 706-712, 2013
18. Andreyev HJ, Norman AR, Cunningham D, *et al*: Kirsten ras mutations in patients with colorectal cancer: the multicenter 'RASCAL' study. J Natl Cancer Inst 90: 675-684, 1998.
19. Andreyev HJ, Norman AR, Cunningham D, *et al*: Kirsten ras mutations in patients with colorectal cancer: the 'RASCAL II' study. Br J Cancer 85: 692-696, 2001.
20. Barault L, Veyrie N, Jooste V, *et al*: Mutations in the RAS-MAPK, PI(3)K (phosphatidylinositol-3-OH kinase) signaling network correlate with poor survival in a population-based series of colon cancers. Int J Cancer 122: 2255-2259, 2008.
21. Ogino S, Nosho K, Kirkner GJ, *et al*: PIK3CA mutation is associated with poor prognosis among patients with curatively resected colon cancer. J Clin Oncol 27: 1477-1484, 2009.
22. Carpten JD, Faber AL, Horn C, *et al*: A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. Nature 448: 439-444, 2007.
23. Sartore-Bianchi A, Martini M, Molinari F, *et al*: PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. Cancer Research 69: 1851-1857, 2009.
24. Ince WL, Jubb AM, Holden SN, *et al*: Association of k-ras, b-raf, and p53 status with the treatment effect of bevacizumab. J Natl Cancer Inst 97: 981-989, 2005.
25. Hurwitz HI, Yi J, Ince W, *et al*: The clinical benefit of bevacizumab in metastatic colorectal cancer is independent of K-ras mutation status: analysis of a phase III study of bevacizumab with chemotherapy in previously untreated metastatic colorectal cancer. Oncologist 14: 22-28, 2009.
26. Richman SD, Seymour MT, Chambers P, *et al*: KRAS and BRAF mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: results from the MRC FOCUS trial. J Clin Oncol 27: 5931-5937, 2009.
27. Souglakos J, Philips J, Wang R, *et al*: Prognostic and predictive value of common mutations for treatment response and survival in patients with metastatic colorectal cancer. Br J Cancer 101: 465-472, 2009.