RNF43 mutation is associated with aggressive tumor biology along with *BRAF* V600E mutation in right-sided colorectal cancer

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Abstract. Right-sided colorectal cancer (RCRC) demonstrates worse survival outcome compared with left-sided CRC (LCRC). Recently, the importance of *RNF43* mutation and *BRAF* V600E mutation has been reported in the serrated neoplasia pathway, which is one of the precancerous lesions in RCRC. It was hypothesized that the clinical significance of *RNF43* mutation differs according to primary tumor sidedness. To test this hypothesis, the clinicopathological characteristics and survival outcome of patients with *RNF43* mutation in RCRC and LCRC were investigated. Stage I-IV CRC patients (n=201) were analyzed. Genetic alterations including *RNF43* using a 415-gene panel were investigated. Clinicopathological characteristics between *RNF43* wild-type and *RNF43* mutant-type were analyzed. Moreover, *RNF43* mutant-type was classified according to primary tumor sidedness, i.e., right-sided *RNF43* mutant-type or left-sided *RNF43* mutant-type, and the clinicopathological characteristics between the two groups were compared. *RNF43* mutational prevalence, spectrum and frequency between our cohort and TCGA samples were compared. *RNF43* mutation was observed in 27 out of 201 patients (13%). Multivariate analysis revealed that age (≥65), absence of venous invasion, and *BRAF* V600E mutation were independently associated with *RNF43* mutation. Among the 27 patients with *RNF43* mutation, 12 patients were right-sided *RNF43* mutant-type and 15 left-sided *RNF43* mutant-type. Right-sided *RNF43* mutant-type was significantly associated with histopathological grade 3, presence of lymphatic invasion, *APC* wild, *BRAF* V600E mutation, microsatellite instability-high (MSI-H), and *RNF43* nonsense/frameshift mutation compared with left-sided *RNF43* mutant-type. Similarly, *RNF43* nonsense/frameshift mutations were more frequently observed in RCRC compared with LCRC in the TCGA cohort (P=0.042). Right-sided *RNF43* mutant-type exhibited significantly worse overall survival than *RNF43* wild-type and left-sided *RNF43* mutant-type (P=0.001 and P=0.023, respectively) in stage IV disease. *RNF43* mutation may be a distinct molecular subtype which is associated with aggressive tumor biology along with *BRAF* V600E mutation in RCRC.

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Introduction

Primary tumor sidedness has prognostic and predictive value in metastatic colorectal cancer (CRC), and has thus emerged as a new biomarker (1,2). Several analyses revealed that right-sided colorectal cancer (RCRC) exhibited significantly worse prognosis than left-sided colorectal cancer (LCRC) (3-5), and anti-EGFR therapy clearly benefitted patients with LCRC, whereas patients with RCRC derived limited benefit (6-10). However, the mechanism of the differences between RCRC and LCRC has not been fully elucidated.

RCRC and LCRC have different clinicopathological and molecular characteristics. RCRC is generally characterized by being more common in women, and associated with Lynch syndrome, sessile serrated adenoma/polyp (SSA/P), mitogen-activated protein kinase signaling, microsatellite instability-high (MSI-H), deficiency of mismatch repair genes, CpG island methylation, and *KRAS* and *BRAF* V600E mutations (11-15). LCRC is more common in men, and associated with familial adenomatous polyposis syndrome, traditional serrated adenoma (TSA), chromosomal instability, *ERBB1* and *ERBB2* amplifications, and *APC*, *p53*, and *NRAS* mutations (11-15). Based on these clinicopathological and molecular

differences, primary tumor sidedness is considered to be associated with prognosis and efficacy of targeted therapy.

Mutations in *RNF43* have been reported in several solid tumors, such as colorectal (16-18), gastric (19), pancreatic (20), ovarian (21), and endometrial (22) cancers. *RNF43* encodes a RING-type E3 ubiquitin ligase, and the protein is predicted to contain a transmembrane domain, a protease-associated domain, an ectodomain, and a cytoplasmic RING domain (23). Expression of RNF43 results in increased ubiquitination of frizzled receptors, and an alteration in their subcellular distribution, resulting in reduced surface levels of these receptors. RNF43 is considered to negatively regulate WNT signaling, and functions as a tumor suppressor. Loss of RNF43 results in decrease or lack of degradation of frizzled receptors, with an enhancement of WNT signaling. In cancer cells, inactivation of RNF43 through *RNF43* mutation is one of the causes of permanent activation of the WNT signaling pathway (23).

Serrated neoplasia, which is a precancerous lesion of CRC, is associated with primary tumor sidedness: SSA/P is associated with RCRC, while TSA is associated with LCRC (24). Recently, several studies revealed the importance of *RNF43* mutation in the serrated neoplasia pathway, i.e., *RNF43* mutation was associated with serrated neoplasia pathway such as SSA/P (25) and TSA (26,27). Moreover, it has been reported that *RNF43* mutation in serrated neoplasia is associated with *BRAF* V600E mutation (17), which is recognized as one of the characteristics of RCRC and a significant negative prognostic factor in metastatic CRC (1,2). Collectively, it was surmised that *RNF43* mutation may play different roles in RCRC and LCRC. Recently, it has been reported that *RNF43* mutations contribute to tumorigenesis in RCRC (18). However, to date, clinical significance of *RNF43* mutation have not been fully investigated according to primary tumor sidedness. It was hypothesized that the clinical significance of *RNF43* mutations differ between RCRC and LCRC. To test this hypothesis, the clinicopathological characteristics and survival outcome of patients with *RNF43* mutation in RCRC and LCRC were investigated.

Materials and methods

Patients. This retrospective study was approved by the Ethics Committee of the Niigata University School of Medicine, and performed in accordance with the Helsinki Declaration (G2015-0816). All methods were performed in accordance with the relevant guidelines and regulations, and written informed consent was obtained from the patients. A total of 201 Japanese patients (117 male and 84 female patients; median age 65 years old; range, 30-94 years) with stage I-IV CRC according to AJCC, 7th edition (28) who underwent a primary tumor resection between January 2009 and December 2015 at the Niigata University Medical and Dental Hospital or Niigata Cancer Center Hospital were included in this study. The median follow-up period was 34 months (range, 1-92 months). Patients diagnosed with adenocarcinoma were included. Patients under 18 years old were excluded. Patients with synchronous double primary CRC or other active concurrent malignant diseases, inflammatory bowel disease or familial adenomatous polyposis were excluded. No patient had received neoadjuvant chemoradiation. Typically, chemotherapy was administered according to the Japanese Society for Cancer of the Colon and Rectum (JSCCR) guidelines (29). Adjuvant chemotherapy, including fluorouracil or its derivatives ± oxaliplatin, was usually administered in stage III patients for six months. For patients with unresectable metastatic diseases, molecular targeted therapy was administered according to RAS mutational status.

In the present analysis, *RNF43* mutational prevalence, spectrum and frequency between our cohort and TCGA samples were compared. The mutation information for the TCGA CRC-sequenced samples (n=489) was obtained from the cBioPortal (https://www.cbioportal.org/) (30) to assess mutation frequency.

Comprehensive genomic sequence analysis of primary tumors. As previously described (15,31-34), formalin-fixed, paraffin-embedded (FFPE) samples were used for next-generation sequencing (NGS), and genetic alterations, including *RNF43*, were evaluated. Briefly, hematoxylin and eosin-stained sections were used to assess tumor content, to ensure that >50% tumor content was present. Where applicable, unstained sections were macro-dissected to enrich for tumor content. DNA was extracted using a BioStic FFPE Tissue DNA Isolation Kit (Mo Bio Laboratories, Inc.). All sample preparation, NGS, and bioinformatics analysis were performed in a CLIA/CAP-accredited laboratory (KEW, Inc.). DNA fragment libraries (50-150 ng) were prepared and enriched for the 415-gene panel with CANCERPLEX Version 3.0 (KEW, Inc.). An average 500X sequencing depth was achieved using Illumina MiSeq or NextSeq platforms. A proprietary bioinformatics platform and knowledge base were used to process genomic data and to identify multiple genomic abnormalities, including SNPs, small indels, copy number variation, and translocations. An allelic fraction threshold of 10% was used for SNPs and indels, and thresholds of >2.5-fold for gain, and 0.5-fold for loss, were used. Tumors were assessed for the presence of MSI on the basis of an extended loci panel. In addition to the Bethesda panel (35), a collection of 950 regions consisting of tandem repeats of one, two or three nucleotides with a minimum length of 10 bases was used (31). Tumor mutational burden was calculated as the number of non-synonymous mutations per megabase of sequence in the panel (panel size=1.3 Mb).

RNF43 status and clinicopathological characteristics. The 201 patients were classified into *RNF43* wild-type or *RNF43* mutant-type; moreover, *RNF43* mutant-type were subdivided into right-sided *RNF43* mutant-type or left-sided *RNF43* mutant-type according to primary tumor sidedness. Primary tumor location was determined by operative findings. Cancer in the cecum, ascending colon, hepatic flexure, or transverse colon was classified as RCRC; while cancer in the splenic flexure, descending colon, sigmoid colon, rectosigmoid, or rectum was classified as LCRC (15).

Statistical analysis. Statistical analyses were performed with IBM SPSS Statistics 22 (IBM Japan, Inc.). Fisher's exact test was used to evaluate the associations between *RNF43* status and clinicopathological characteristics. To clarify clinicopathological characteristics which were independently associated with *RNF43* mutation, factors with a P-value of <0.10 in univariate analyses were entered into a multivariate

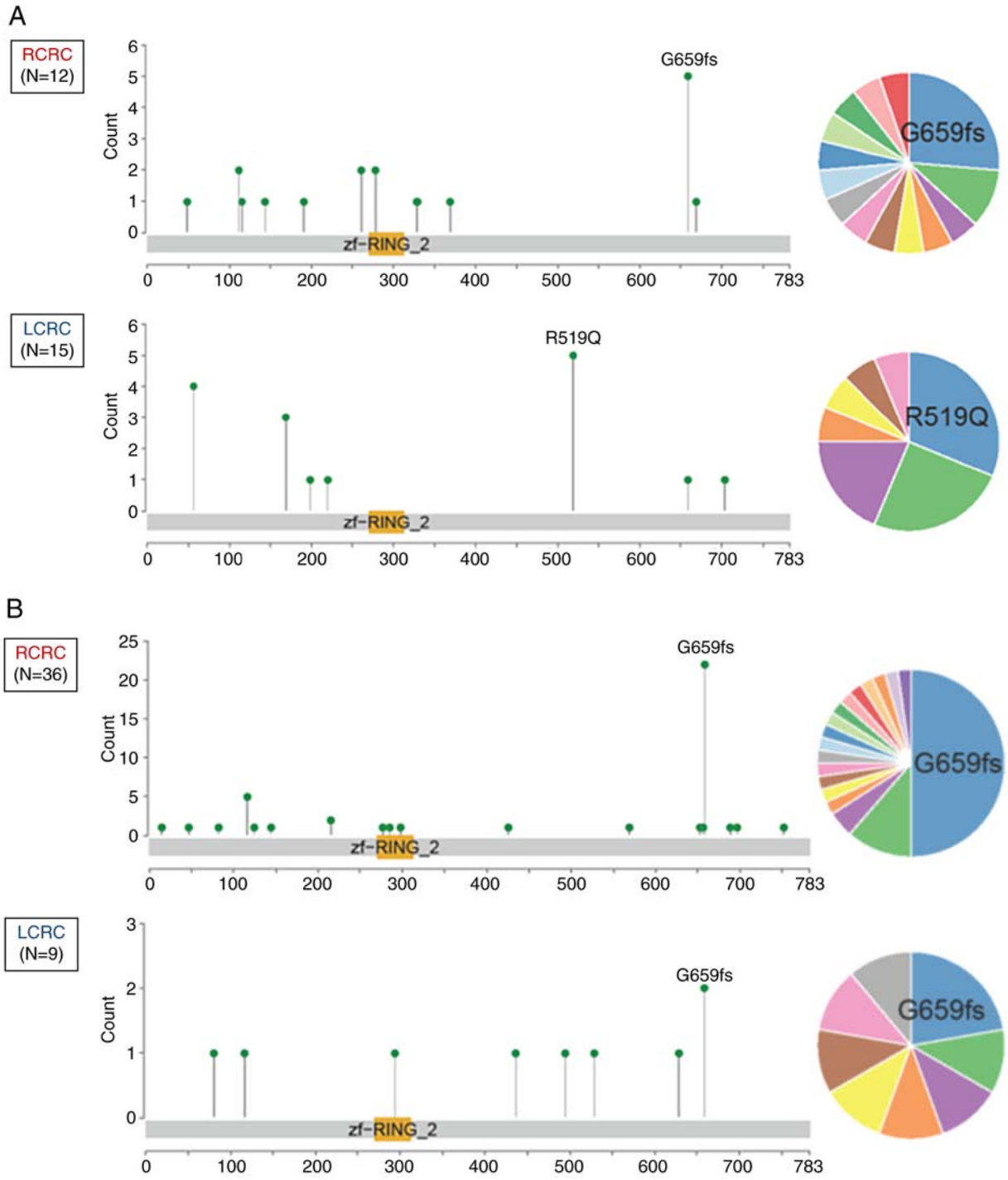

Figure 1. The location and frequency of *RNF43* mutations according to primary tumor sidedness. (A) Japanese samples; (B) TCGA samples. TCGA, The Cancer Genome Atlas.

analysis. Logistic analysis was performed to identify factors that were independently associated with *RNF43* mutation. Five-year overall survival (OS) rates were estimated using the Kaplan-Meier method. The log-rank test was used to assess for significant differences between subgroups. P-values <0.05 were considered to indicate statistically significant differences.

Results

Alteration of RNF43 in Japanese CRC. To date, there has been no studies regarding genetic alterations of *RNF43* among Japanese CRC patients; hence, the genetic alterations of *RNF43* were evaluated and compared with The Cancer Genome Atlas (TCGA) data (https://www.cbioportal.org/). *RNF43* nonsense/frameshift mutation was more frequently observed in RCRC compared with LCRC in both of the Japanese cohorts (P<0.001; Figs. 1A and 2A) and TCGA samples (P=0.042; Figs. 1B and 2B).

Clinicopathological characteristics in relation to RNF43 mutation status. The 415-gene panel assessment successfully detected genetic alterations in all 201 patients. The 415-gene

Table I. *RNF43* gene status and other clinicopathological characteristics in colorectal cancer.

	RNF43 gene status			Multivariate	
Variables	Wild N (%)	Mutant N (%)	Univariate P-value	Odds ratio (95% CI)	P-value
Age (years)					
<65	94 (46)	6 (3)	**0.003**	1	
≥65	80 (40)	21 (10)		3.04 (1.03-8.90)	**0.042**
Sex					
Male	108 (53)	9 (4)	**0.006**		
Female	66 (33)	18 (9)			
Location					
Right side	44 (22)	12 (6)	0.062		
Left side	130 (65)	15 (7)			
Tumor size (mm)					
<50	75 (37)	13 (6)	0.679		
≥50	99 (49)	14 (7)			
pT category					
T1, 2	20 (10)	4 (2)	0.539		
T3, 4	154 (76)	23 (11)			
Histopathological grading					
G1, 2	128 (63)	19 (9)	0.816		
G3	46 (23)	8 (4)			
Lymphatic invasion					
Absence	65 (32)	14 (7)	0.203		
Presence	109 (54)	13 (6)			
Venous invasion					
Absence	35 (17)	13 (6)	**0.003**	1	
Presence	139 (69)	14 (7)		0.18 (0.06-0.52)	**0.002**
pN category					
N0	49 (24)	10 (5)	0.362		
N1, 2	125 (62)	17 (8)			
cM category					
M0	72 (36)	18 (9)	**0.021**		
M1	102 (51)	9 (4)			
APC					
Wild-type	29 (14)	9 (4)	0.061		
Mutant	145 (72)	18 (9)			
KRAS					
Wild-type	105 (52)	21 (10)	0.091		
Mutant	69 (34)	6 (3)			
BRAF V600E					
Wild-type	171 (85)	17 (9)	**<0.001**	1	
Mutant	3 (1)	10 (5)		45.68 (9.76-213.81)	**<0.001**
MSI					
MSI-H	7 (3)	8 (4)	**<0.001**		
MSS	167 (84)	19 (9)			

Fisher's exact test. CI, confidence interval; MSI, microsatellite instability; MSI-H, microsatellite instability-high. Bold indicates P<0.05.

panel assessment revealed that 174 (87%) patients were *RNF43* wild-type and 27 (13%) patients were *RNF43* mutant-type. *RNF43* mutant-type was significantly associated with age (≥65; P=0.003), females (P=0.006), absence of venous

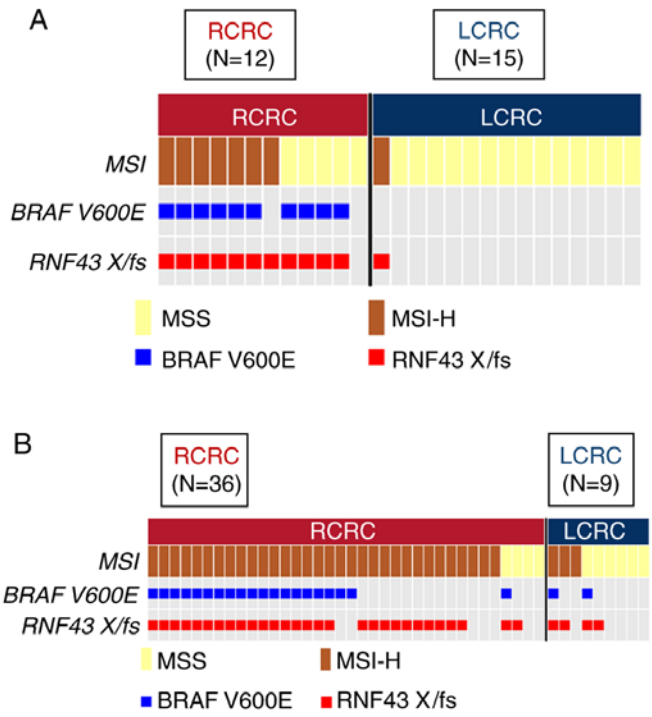

Figure 2. Oncoprint of right-sided *RNF43* mutant-type and left-sided *RNF43* mutant-type. (A) Japanese samples; (B) TCGA samples. TCGA, The Cancer Genome Atlas.

invasion (P=0.003), absence of distant metastasis (P=0.021), *BRAF* V600E mutation (P<0.001), and MSI-H (P<0.001), and multivariate analysis revealed that age (≥65), absence of venous invasion, and *BRAF* V600E mutation were independently associated with *RNF43* mutation (Table I).

Genetic alterations of the MAPK pathway other than BRAF V600E mutation in RNF43 mutant-type. Seventeen of the 27 patients with *RNF43* mutant-type had no *BRAF* V600E mutation. Nine of the 17 patients had mutations other than *BRAF* V600E in the MAPK pathway: 6 patients had *KRAS* mutation, 3 patients had *BRAF* non-V600E mutation; however, no patient had *NRAS* mutation.

RNF43 mutant-type according to primary tumor sidedness. Among the 27 patients with *RNF43* mutation, 12 patients were right-sided *RNF43* mutant-type and 15 left-sided *RNF43* mutant-type. As revealed in Fig. 2A, 11 of the 12 right-sided *RNF43* mutant-type had nonsense/frameshift mutations, while 14 of 15 left-sided *RNF43* mutant-type had missense mutations. Right-sided *RNF43* mutant-type was significantly associated with histopathological grade 3 (P=0.008), lymphatic invasion (P=0.021), *APC* wild (P=0.003), *BRAF* V600E mutation (P<0.001), MSI-H (P=0.008), and *RNF43* nonsense/frameshift mutation (P<0.001) compared with left-sided *RNF43* mutant-type (Table II; Fig. 2A).

Overall survival in relation to RNF43 status and primary tumor sidedness. In 90 patients with stage I-III disease, *RNF43* mutation was not a significant prognostic factor for 5 year OS (Fig. 3A), and primary tumor sidedness was not associated with *RNF43* mutant-type.

Table II. Clinicopathological characteristics according to primary tumor sidedness in *RNF43* mutant colorectal cancer.

Variables	Primary tumor sidedness		P-value
	Right-sided N (%)	Left-sided N (%)	
Age (years)			
<65	1 (4)	5 (18)	0.182
≥65	11 (40)	10 (37)	
Sex			
Male	3 (11)	6 (22)	0.683
Female	9 (33)	9 (33)	
Tumor size (mm)			
<50	5 (18)	8 (29)	0.704
≥50	7 (26)	7 (26)	
pT category			
T1, 2	1 (4)	3 (11)	0.605
T3, 4	11 (40)	12 (44)	
Histopathological grading			
G1, 2	5 (18)	14 (52)	**0.008**
G3	7 (26)	1 (4)	
Lymphatic invasion			
Absence	3 (11)	11 (40)	**0.021**
Presence	9 (33)	4 (15)	
Venous invasion			
Absence	4 (15)	9 (33)	0.252
Presence	8 (30)	6 (22)	
pN category			
N0	3 (11)	7 (26)	0.424
N1, 2	9 (33)	8 (30)	
cM category			
M0	8 (30)	10 (37)	0.999
M1	4 (15)	5 (18)	
APC			
Wild-type	8 (30)	1 (4)	**0.003**
Mutant	4 (15)	14 (52)	
KRAS			
Wild-type	11 (40)	10 (37)	0.182
Mutant	1 (4)	5 (18)	
BRAF V600E			
Wild-type	2 (7)	15 (55)	**<0.001**
Mutant	10 (37)	0 (0)	
MSI			
MSI-H	7 (26)	1 (4)	**0.008**
MSS	5 (18)	14 (52)	
Variants of *RNF43*			
Nonsense or frameshift	11 (40)	1 (4)	**<0.001**
Missense	1 (4)	14 (52)	

Fisher's exact test. MSI, microsatellite instability; MSI-H, microsatellite instability-high. Bold indicates P<0.05.

In 111 patients with stage IV disease, *RNF43* mutation was not a significant prognostic factor for OS (Fig. 3B).

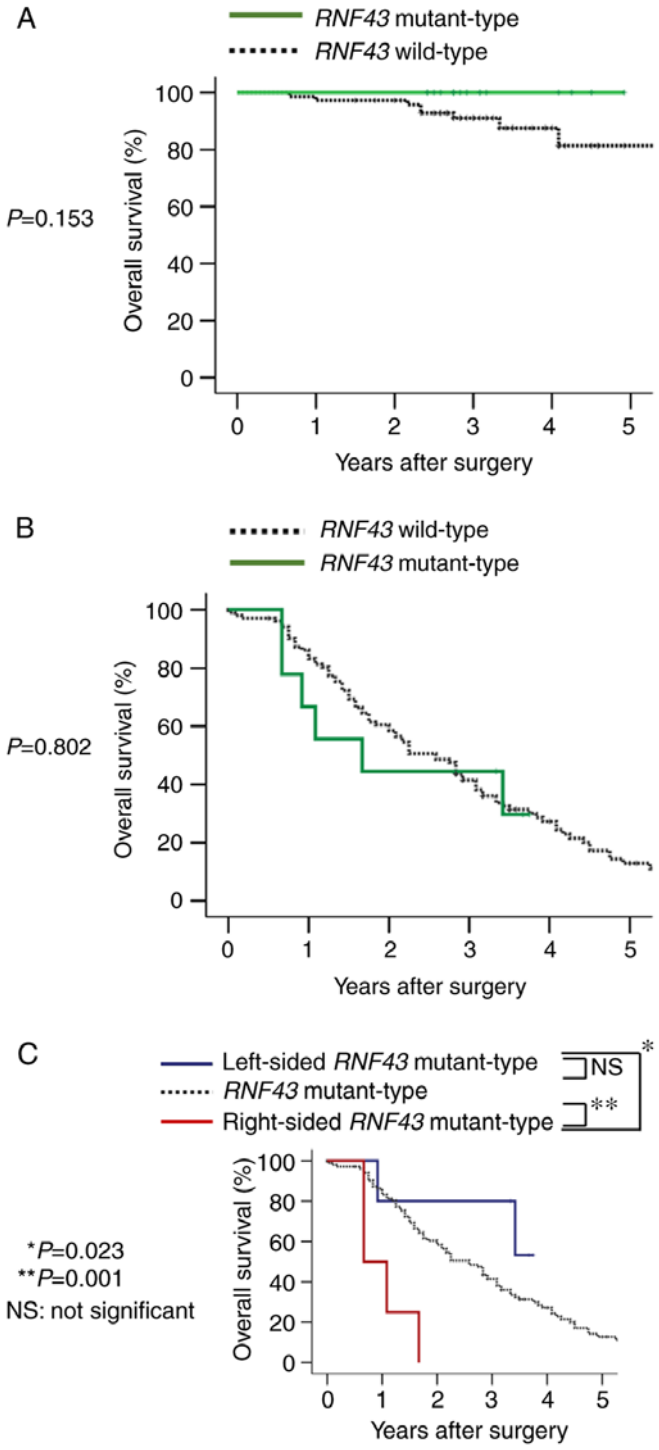

Figure 3. Overall survival according to *RNF43* mutation status and primary tumor sidedness. (A) Overall survival of *RNF43* wild-type and *RNF43* mutant-type in stage I-III colorectal cancer. (B) Overall survival of *RNF43* wild-type and *RNF43* mutant-type in stage IV colorectal cancer. (C) Overall survival of *RNF43* wild-type, right-sided *RNF43* mutant-type, and left-sided *RNF43* mutant-type in stage IV colorectal cancer.

However, when *RNF43* mutant-type was subdivided into right-sided *RNF43* mutant-type or left-sided *RNF43* mutant-type according to primary tumor sidedness, right-sided *RNF43* mutant-type exhibited significantly worse overall survival than *RNF43* wild-type and left-sided *RNF43* mutant-type (P=0.001 and P=0.023, respectively; Fig. 3C). Regarding variants of *RNF43* mutations, all right-sided *RNF43* mutant-type had nonsense mutation (R145X) or frameshift mutation (P192fs, S262fs, G659fs), while all left-sided *RNF43* mutant-type had missense mutations (T58S, W200C, R221W, R519Q; Table III). All the four right-sided *RNF43* mutant-type were older in age (≥65), females, and *BRAF* V600E mutant-type. Three of four patients with right-sided *RNF43* mutation had two or more metastatic sites; conversely, all five patients with left-sided *RNF43* mutation had one metastatic site. While all patients with right-sided *RNF43* mutant-type succumbed to their cancer, three of the five patients with left-sided *RNF43* mutant-type were alive at the final follow-up (Table III).

Discussion

This analysis has three main findings regarding *RNF43* mutations in CRC. Firstly, most of *RNF43* mutations in RCRC were nonsense or frameshift mutations, while those in LCRC were missense mutations. Secondly, right-sided *RNF43* mutant-type was significantly associated with histopathological grade 3 and *BRAF* V600E mutation. Thirdly, right-sided *RNF43* mutant-type exhibited significantly worse OS than left-sided *RNF43* mutant-type. These results indicated that right-sided *RNF43* mutant-type is one of the clinically important subtypes in CRC, and *RNF43* nonsense/frameshift mutations, along with *BRAF* V600E mutation, may be a possible cause of worse prognosis of RCRC.

In this analysis, it was revealed that 13% of the Japanese CRC patients in this study had *RNF43* mutations, while 9% of patients in the TCGA cohort had *RNF43* mutations (36,37). Recently, several studies have revealed the role of *RNF43* mutations in the serrated neoplastic pathway of CRC. Hashimoto *et al* reported that WNT pathway gene mutations, including *RNF43* mutation, were more common in SSA/P with dysplasia than in SSA/P without dysplasia, and suggested that WNT pathway gene mutations are involved in the development of dysplasia in SSA/P (25). Tsai *et al* reported the incidence of *RNF43* mutation in SSA/P (10%) and TSA (28%), and stated that *RNF43* mutation is an early and specific molecular aberration in the serrated neoplasia pathway (26). Yan *et al* reported *RNF43* germline and somatic mutation along with *BRAF* V600E mutation in the serrated neoplasia pathway (16). However, the clinical significance of *RNF43* mutation has not been fully elucidated; hence, the clinicopathological characteristics of *RNF43* mutation were investigated, with a focus on the association between *RNF43* mutation and primary tumor sidedness.

To the best of our knowledge, this is the first study which investigated the survival outcome of *RNF43* mutant-type according to primary tumor sidedness in CRC. Previous studies have reported that hotspot mutations, mainly frameshift (R117fs and G659fs), are found in microsatellite-instable SSA/P and CRC (23). In this analysis, 201 patients with stage I-IV CRC were investigated, and it was revealed that 11 out of 12 right-sided *RNF43* mutant-type had nonsense/frameshift mutations, while 14 out of 15 left-sided *RNF43* mutant-type had missense mutations. Although RNF43 protein expression was not investigated, *RNF43* nonsense/frameshift mutation may be a cause of loss of function of RNF43 protein. It is speculated that *RNF43* nonsense/frameshift mutation

Table III. Clinical course of *RNF43* mutant-type patients with Stage IV disease.

Genetic alteration	Age	Sex	Primary tumor location	*KRAS* status	*BRAF* status	MSI status	Tumor mutational burden	Initial metastatic sites	Treatment	Survival status (months after primary tumor resection)
S262fs	71	F	Right	Wild	V600E	MSS	19	Liver, Lung, Spleen, Peritoneum	R2 resection (Primary) → FOLFOX + Bmab → FOLFIRI	Dead (8 months)
R145X	66	F	Right	Wild	26_34del, V600E	MSS	19	Para-aortic lymph node	R0 resection (Primary and Para-aortic LN) → Lung, LN recurrence → FOLFOX + Bmab	Dead (13 months)
P192fs	80	F	Right	Wild	V600E	MSS	18	Lung	R2 resection (Primary) → XELOX + Bmab	Dead (20 months)
G659fs	78	F	Right	Wild	V600E	MSI-H	48	Liver, Peritoneum	R2 resection (Primary) → FOLFOX + Pmab	Dead (8 months)
R519Q	35	F	Left	Wild	Wild	MSS	10	Liver	R2 resection (Primary) → FOLFOX + Bmab → R0 resection (Liver) → Liver and lung recurrence → FOLFOX + Bmab → FOLFIRI + Pmab	Alive (44 months)
R519Q	86	M	Left	Wild	Wild	MSS	11	Lung	R2 resection (Primary) → Xeloda → XELOX + Bmab → IRIS + Pmab	Alive (45 months)
W200C	70	F	Left	Wild	D594G	MSS	19	Liver	R2 resection (Primary) → R0 resection (Liver) → Liver recurrence → R0 resection (Liver)	Alive (40 months)
R221W	77	M	Left	Wild	Wild	MSS	12	Liver	R2 resection (Primary) → XELOX → IRIS → Pmab	Dead (11 months)
T58S	75	F	Left	Wild	Wild	MSS	11	Liver	R2 resection (Primary) → XELOX + Bmab → R0 resection (Liver) → Lung recurrence	Dead (41 months)

FOLFOX = 5FU + Leucovorin + Oxaliplatin; FOLFIRI = 5FU + Leucovorin + Irinotecan; XELOX = Xeloda + Oxaliplatin; IRIS = Irinotecan + S-1. Bmab, Bevacizumab; Pmab, Panitumumab; MSS, microsatellite stable.

can become a cause of stimulation of the WNT signaling pathway, and is associated with the aggressive tumor biology of RCRC.

Approximately 5 to 9% patients with CRC have *BRAF* V600E mutation, and *BRAF* V600E mutation is recognized as a distinct molecular subtype of CRC (1,2). Multiple studies have revealed that the *BRAF* V600E mutation is associated with poor prognosis in metastatic CRC (38,39), as well as poor response to anti-EGFR therapy in later lines of therapy (40,41). In the present study, it was revealed that 10 out of 12 right-sided *RNF43* mutant-type had *BRAF* V600E mutation. It is surmised that both *RNF43* and *BRAF* V600E mutations are important for the tumor biology of right-sided *RNF43* mutant-type; i.e., *RNF43* nonsense/frameshift mutation, along with *BRAF* V600E mutation, induce enhancement of both the WNT and MAPK signaling pathways, resulting in a worse prognosis in right-sided *RNF43* mutant-type.

In stage IV disease, it was revealed that right-sided *RNF43* mutant-type exhibited significantly worse OS than left-sided *RNF43* mutant-type, and all patients with right-sided *RNF43* mutant-type succumbed to their cancer. These results suggest that right-sided *RNF43* mutant-type is a distinct subtype that has potentially worse prognosis. We consider that *RNF43* mutation should be treated differently according to primary tumor sidedness, since the clinicopathological characteristics and survival outcomes differ between right-sided *RNF43* mutant-type and left-side *RNF43* mutant-type. Thus, how should this dismal molecular subtype 'right-sided *RNF43* mutant-type' be treated? At present, right-sided *RNF43* mutant-type may be treated the same as *BRAF* V600E mutant-type (1,2), since it was revealed that most right-sided *RNF43* mutant-type cases had *BRAF* V600E mutation in this analysis. In the future, WNT signaling plus BRAF inhibitors may be applied for right-sided *RNF43* mutant-type (ClinicalTrials.gov Identifier: NCT02278133).

This analysis has some limitations. First, this retrospective analysis was performed at two institutions. Second, it included a small number of patients; specifically, the study had only 90 patients with Stage I-III disease. Future analysis should include a larger number of patients with CRC from large-scale multi-institutional studies or a cancer registry. It is speculated that the microbiome may be associated with tumor carcinogenesis and phenotype, and certain bacteria may be associated with genetic alterations in CRC. For example, it has been reported that *Fusobacterium nucleatum* is enriched in tumor tissue of MSI-H CRC (42,43). Although we do not have data linking the microbiome to the results of our study at present, we plan to investigate the relationship between genetic alteration of right-sided CRC and the patient microbiome. Collectively, this analysis is important for clarifying the clinicopathological characteristics and prognosis of *RNF43* mutant-type according to primary tumor sidedness, and facilitating the research of future treatment strategies.

In conclusion, clinicopathological characteristics and survival outcome of patients with *RNF43* mutation may differ between RCRC and LCRC. In RCRC, *RNF43* mutation may be a small, but distinct molecular subtype that is associated with aggressive tumor biology along with *BRAF* V600E mutation. Future preclinical and clinical studies may have to focus on *RNF43* mutation to improve survival outcome in CRC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YS and AM provided substantial contributions to the design and interpretation of data, and drafting of the article. MaeN, HO, YoT, MasN, HK, YH, HI, MNag, HN, SM, YaT, and TW provided substantial contributions to the acquisition of clinical data and interpretation of data. YL and SO provided substantial contributions to the statistical analysis of the data and creation of the figures. TW critically revised the work and provided final approval of article. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This retrospective study was approved by the Ethics Committee of the Niigata University School of Medicine, and performed in accordance with the Helsinki Declaration (G2015-0816). All methods were performed in accordance with the relevant guidelines and regulations, and written informed consent was obtained from the patients.

Patient consent for publication

Not applicable.

Competing interests

The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.

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