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Significance of Plasma *UCA1* for Predicting Colorectal Cancer and BRAF Mutation Status

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Abstract. *Background: Clinical significance of plasma urothelial carcinoma associated 1 (UCA1) in patients with colorectal cancer (CRC) remains unclear. This study investigated the usefulness of plasma UCA1 as a biomarker in patients with CRC. Materials and Methods: UCA1 levels were measured in the plasma and tissue from patients with CRC by quantitative polymerase chain reaction. Relationships between plasma UCA1 and clinicopathological features were examined. Results: Plasma UCA1 levels were significantly lower in patients with CRC than in healthy volunteers. UCA1 expression in B-Raf proto-oncogene serine/threonine kinase (BRAF)-mutant CRC tissue was also lower than that in non-cancerous tissue, although it was higher in CRC with wild-type BRAF. In right-sided CRC, a lower plasma UCA1 level was associated with pT4 and BRAF mutation. In contrast, in left-sided CRC, higher plasma UCA1 was associated with pT4 and pStage 3b-4. Conclusion: Plasma UCA1 is a useful biomarker for CRC detection and predicting clinicopathological features, particularly BRAF mutation.*

Colorectal cancer (CRC) is the third most common cancer worldwide and has the second highest mortality rate (1). The cancer-related molecular mechanisms of CRC were recently elucidated, and less invasive surgical therapies followed by many drug therapies are now becoming available (2-4). Therefore, the number of CRC-related deaths may be reduced by early-stage diagnosis and the initiation of appropriate curative treatments. Moreover, the identification and elucidation of other novel molecular mechanisms of CRC will improve the prognosis of patients.

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Key Words: UCA1, colorectal cancer, BRAF mutation.

Long non-coding RNAs (lncRNAs) are of more than 200 nucleotides in length and classified as a family of non-protein-coding RNAs. They play a role in the development of various cancer types (5-10). Urothelial carcinoma-associated 1 (*UCA1*) is a lncRNA that was initially identified in bladder cancer (11), and its oncogenic roles and usefulness as a diagnostic marker have been confirmed (12, 13). Recent studies reported that *UCA1* was up-regulated in CRC tissue, and high *UCA1* expression in cancer tissue was associated with a poorer prognosis in patients with CRC (12-14).

Many non-coding RNAs, including lncRNAs, have been detected in the plasma of patients with cancer and identified as leading candidates for liquid biopsy (7, 15, 16). However, only a few studies have examined the level of circulating *UCA1* in CRC. Therefore, the significance of the circulating *UCA1* in relation to clinicopathological features and the prognosis remains unclear (7, 12-14).

In the present study, we examined the plasma *UCA1* level in patients with CRC and investigated its relationship with clinicopathological features. *UCA1* expression was also assessed in CRC tissue samples to clarify the relationship with the B-Raf proto-oncogene serine/threonine kinase (*BRAF*) mutation status.

Materials and Methods

Patients and samples. Pre-operative plasma samples were collected from 76 patients with CRC who underwent radical resection of the primary lesion at Kyoto Prefectural University of Medicine between 2015 and 2019. Some patients with stage 4 disease had residual liver or lung metastasis after surgery. Twenty control samples were collected from healthy volunteers (HVs) who underwent surgery for non-cancer diseases, for example, inguinal hernia and cholelithiasis, under general anesthesia. Relevant clinical and survival data were available for all patients with CRC. Some paired cancer and non-cancerous mucosal samples were also collected from the surgically resected specimens of patients whose plasma *UCA1* levels were examined.

Macro- and microscopic classifications of cancer were based on the ninth edition of the Japanese Classification of Colorectal, Appendiceal, and Anal Carcinoma (17). In the present study, right-

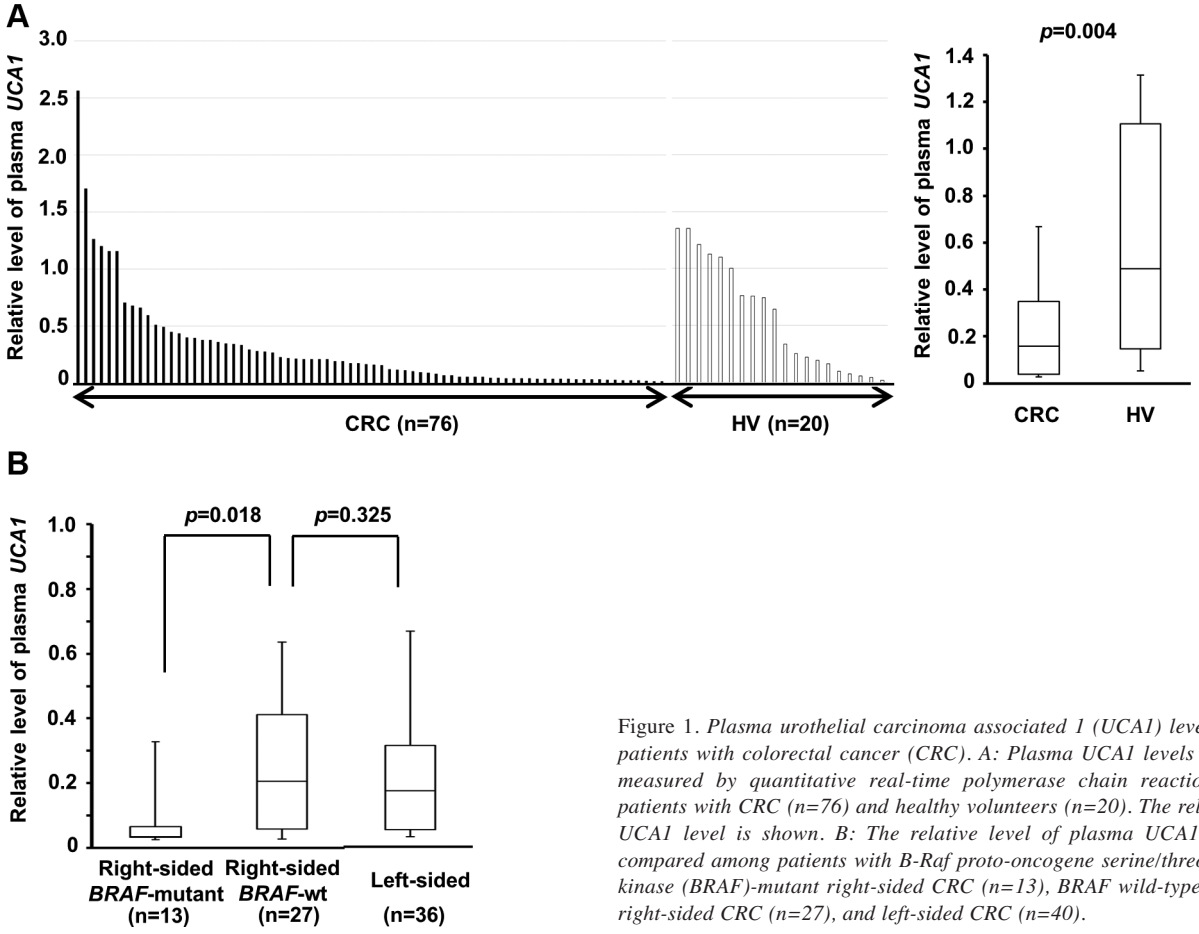

Figure 1. *Plasma urothelial carcinoma associated 1 (UCA1) levels in patients with colorectal cancer (CRC). A: Plasma UCA1 levels were measured by quantitative real-time polymerase chain reaction in patients with CRC (n=76) and healthy volunteers (n=20). The relative UCA1 level is shown. B: The relative level of plasma UCA1 was compared among patients with B-Raf proto-oncogene serine/threonine kinase (BRAF)-mutant right-sided CRC (n=13), BRAF wild-type (wt) right-sided CRC (n=27), and left-sided CRC (n=40).*

sided CRC consisted of cancer of the cecum and ascending and transverse colon up to the splenic flexure, while left-sided CRC comprised those of the descending and sigmoid colon and rectum.

Ethics statement. Ethical approval was granted by the Faculty of Science Ethics Committee at the Kyoto Prefectural University of Medicine, and the present study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent on treatments and participation in the present study was obtained from all CRC patients and HVs.

Sample collection and storage. Blood samples were collected from patients with CRC and HVs in ethylenediaminetetra-acetic acid containing tubes (BD Vacutainer; Becton Dickinson and Company, Franklin Lakes, NJ, USA) before surgery under general anesthesia. They were immediately subjected to the preparation of plasma using a 3-spin protocol (350 × *g* for 30 min, 700 × *g* for 5 min, and 1600 × *g* for 5 min) to prevent contamination by cellular fractions. Plasma samples were stored at −80˚C until further analyses.

RNA extraction. Total RNA was extracted from 400 μl of plasma samples using mirVana PARIS Kit (Ambion, Austin, TX, USA), and eluted into 100 μl of pre-heated (95˚C) Elution Solution according to the manufacturer's instructions. For tissue samples, total RNA was extracted from four 15-μm-thick formalin-fixed paraffin-embedded tissue slices (total thickness of 60 μm) using the RecoverAll Total Nucleic Acid Isolation Kit (Ambion). It was finally eluted into 60 μl of Elution Solution according to the manufacturer's instructions. RNA samples were then stored at −80˚C until further processing.

Detection of lncRNA by polymerase chain reaction (PCR). Reverse transcription reaction was performed using the High-Capacity RNA-to-cDNA Kit (Applied Biosystems, Foster City, CA, USA). Nine microliters of total RNA extracted from 400 μl of plasma was used for this reaction. Regarding RNA extracted from tissue samples, the total RNA concentration was adjusted to 100 ng/μl, and the reverse transcription reaction was performed using 5 μl of RNA with High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). These cDNA products were pre-amplified using the TaqPath qPCR Master Mix, CG (Applied Biosystems) and *UCA1* primer of the human TaqMan Gene Expression Assay Kit (Assay ID: Hs05052446_gH; Applied Biosystems).

UCA1 levels were measured in duplicate by quantitative real-time PCR using the *UCA1* primer following the manufacturer's protocol. The quantitative PCR analysis was performed using the Step One Plus Real-time PCR system (Applied Biosystems), and cycle threshold (Ct) values were calculated with Step One Software version 2.2.2 (Applied Biosystems).

Plasma *UCA1* levels were calculated using the ΔCt method relative to the plasma *UCA1* level of one HV who underwent cholecystectomy for gallbladder stones and had no specific medication or previous history. *UCA1* expression in tissue was normalized using the ΔΔCt method relative to the expression of glyceraldehyde-3-phosphate dehydrogenase (Assay ID: Hs02758991_g1; Applied Biosystems) in each sample. Finally, it was reported as a value relative to the expression in non-cancerous tissue of one patient with CRC (CRC160).

Confirmation of the BRAF mutation. In some cases, the *BRAF* gene V600E mutation status had already been analyzed. However, the mutation status of V600E was confirmed by focusing on cases of right-sided CRC using competitive allele-specific TaqMan PCR technology (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The *BRAF*_476_mu (Assay ID: Hs00000111_mu) probe with the corresponding wild-type allele assay *BRAF*_476_wt (Assay ID: Hs00000110_wt) and gene reference assay *BRAF*_rf (Assay ID: Hs00000172_rf; all from Applied Biosystems) were used in the Step One Plus Real-time PCR System (Thermo Fisher Scientific) following the manufacturer's protocol.

Statistical analysis. The Mann–Whitney test or Student's *t*-test was used to compare differences in the plasma *UCA1* levels of unpaired samples or investigate relationships between plasma *UCA1* levels and clinicopathological features. The paired *t*-test and Wilcoxon test were used to evaluate the significance of differences between cancer and non-cancer tissue samples. The median value of plasma *UCA1* was used in the analysis of associations with clinicopathological features. A *p*-value of less than 0.05 was considered to be significant.

Results

Plasma UCA1 level and clinicopathological features. Plasma *UCA1* levels were significantly lower in 76 patients with CRC than in 20 HVs (p=0.004, Figure 1A). Patient backgrounds are shown in Table I. There were 40 and 36 cases of right-sided and left-sided CRC, respectively. Relationships between the plasma *UCA1* level and clinicopathological features in patients with CRC were examined by the median value of plasma *UCA1*. No correlations were found in the 76 cases examined (Table II). However, in right-sided CRC (Table III), a lower plasma *UCA1* level was correlated with *BRAF* V600E mutation (p=0.048) and pT4 factor (p=0.048). On the other hand, in left-side CRC (Table IV), higher plasma *UCA1* levels were correlated with pT4 factor (p=0.03) and pStage 3b-4 (p=0.01).

Relationship between plasma UCA1 level and the BRAF V600E mutation. The plasma *UCA1* level was significantly lower in patients with *BRAF*-mutant right-sided CRC than in those with *BRAF* wild-type cancer (p=0.018), and did not significantly differ between patients with right- and left-sided *BRAF* wild-type CRC (p=0.325, Figure 1B).

UCA1 expression in CRC tissue. To clarify the impact of *BRAF* V600E mutation on *UCA1* expression, *UCA1* expression was examined in 26 paired cancer and non-cancerous tissue samples. The background characteristics of

Table I. *Patient background characteristics (n=76).*

Total	Value
Age, years	
Median (range)	70 (25-94)
Gender, n (%)	
Male	34 (44.7)
Female	42 (55.3)
BRAF gene status, n (%)	
Wild-type	63 (82.9)
Mutant	13 (17.1)
Tumor location, n (%)	
Cecum	4 (5.3)
Ascending colon	25 (32.9)
Transverse colon	11 (14.5)
Descending colon	3 (3.9)
Sigmoidal colon	11 (14.5)
Rectum	22 (28.9)
Macroscopic type, n (%)[a]	
1	5 (6.6)
2	64 (84.2)
3	6 (7.9)
4	1 (1.3)
Tumor size, mm	
Median (range)	45 (4-120)
Histological type, n (%)[a]	
Tub1	42 (55.3)
Tub2	28 (36.8)
Por	1 (1.3)
Sig	1 (1.3)
Muc	4 (5.3)
Lymphatic invasion, n (%)[a]	
0	22 (28.9)
1	31 (40.8)
2	3 (3.9)
3	19 (25)
Unknown	1 (1.3)
Venous invasion, n (%)[a]	
0	22 (28.9)
1	41 (53.9)
2	6 (7.9)
3	6 (7.9)
Unknown	1 (1.3)
pT Factor, n (%)[a]	
1	1 (1.3)
2	2 (2.3)
3	49 (64.5)
4	24 (31.6)
pN Factor, n (%)[a]	
0	26 (34.2)
1	29 (38.2)
2	13 (17.1)
3	8 (10.5)
pStage, n (%)[a]	
1	1 (1.3)
2	23 (30.3)
3	41 (53.9)
4	11 (14.5)

BRAF: B-Raf proto-oncogene serine/threonine kinase; Muc: mucinous adenocarcinoma; Por: poorly differentiated adenocarcinoma; Sig: signet-ring cell carcinoma; Tub1: tubular adenocarcinoma well-differentiated type; Tub2: tubular adenocarcinoma moderately differentiated type. [a]According to the ninth edition of the Japanese Classification of Colorectal Carcinoma (17).

Table II. *Correlations between plasma urothelial carcinoma-associated 1 (UCA1) level and clinicopathological features in patients with colorectal cancer.*

Variable		n (%)	Relative plasma UCA1 level, n (%)		*p*-Value
			≤0.158	>0.158	
Total	n (%)	76	38 (50)	38 (50)	
Age	≤70 Years	39 (51.3)	19 (25.0)	20 (26.3)	0.819
	>70 Years	37 (48.7)	19 (25.0)	18 (23.7)	
Gender	Male	34 (44.7)	20 (26.3)	14 (18.4)	0.165
	Female	42 (55.3)	18 (23.7)	24 (31.6)	
Tumor location	Right (C, A, T)	40 (52.6)	22 (28.9)	18 (23.7)	0.358
	Left (D, S, R)	36 (47.4)	16 (21.1)	20 (26.3)	
Tumor size	≤40 mm	34 (44.7)	17 (22.4)	17 (22.4)	>0.999
	>40 mm	42 (55.3)	21 (27.6)	21 (27.6)	
Histological type[a]	Tub	70 (92.1)	34 (44.7)	36 (47.4)	0.391
	Others	6 (7.9)	4 (5.3)	2 (2.6)	
Lymphatic invasion[a]	Negative	22 (28.9)	14 (18.4)	8 (10.5)	0.146
	Positive	53 (69.7)	24 (31.6)	29 (38.2)	
Venous invasion[a]	Negative	22 (28.9)	12 (15.8)	10 (13.2)	0.335
	Positive	53 (69.7)	26 (34.2)	27 (35.5)	
pT Factor[a]	1-3	52 (68.4)	26 (34.2)	26 (34.2)	>0.999
	4	24 (31.6)	12 (15.8)	12 (15.8)	
pN Factor[a]	Negative	26 (34.2)	14 (18.4)	12 (15.8)	0.629
	Positive	50 (65.8)	24 (31.6)	26 (34.2)	
pStage[a]	1-3a	47 (61.8)	26 (34.2)	21 (27.6)	0.237
	3b-4	29 (38.2)	12 (34.2)	17 (22.4)	

A: Ascending colon; C: cecum; D: descending colon; R: rectum; S: sigmoid colon; T: transverse colon; Tub: tubular adenocarcinoma. [a]According to the ninth edition of the Japanese Classification of Colorectal Carcinoma (17). Note that lymphatic and venous invasion data for one patient are not reported.

these 26 patients are shown in Table V. Eighteen patients with right-sided CRC were included, and *BRAF* V600E mutation was identified in nine patients.

UCA1 expression was significantly higher in cancer tissue than paired non-cancerous tissue (p=0.023, Figure 2A). These differences were observed in those with *BRAF*-wild-type right-sided CRC (p=0.033, Figure 2C) and left-sided CRC (p=0.009, Figure 2D). However, in *BRAF*-mutant right-sided CRC, *UCA1* expression levels were slightly lower in cancer tissue, although not significantly (p=0.189, Figure 2B).

Discussion

UCA1 is strongly expressed in various cancer types (8, 18, 19), such as urinary bladder cancer (11), gastric cancer (20), hepatocellular carcinoma (21), esophageal cancer (22), pancreatic cancer (23), and CRC (12-14). Its overexpression was reported to be correlated with a poor prognosis (8, 19, 24). In CRC, higher *UCA1* expression in tissue and cell lines was correlated with an advanced TNM stage, resistance to chemotherapies, and the progression of malignancies (12, 25-28).

In the present study, the plasma *UCA1* level was significantly lower in patients with CRC than in HVs. They were especially lower in patients with *BRAF*-mutant right-sided CRC than in those with other CRC. Furthermore, *UCA1* expression was slightly lower in *BRAF*-mutant right-sided CRC than the paired non-cancerous tissue, although it was significantly higher in *BRAF* wild-type CRC, regardless of side.

Previous studies reported that *UCA1* expression was significantly higher in CRC tissue than in non-cancer tissue (12-14, 26). Our results are mostly consistent with these findings, but differed for those obtained for patients with *BRAF*-mutant right-sided CRC. The reasons for this difference need to be discussed; however, among the clinicopathological features of patients with CRC investigated in previous studies, the genomic or molecular status was not examined in detail. We are assuming that *BRAF* gene mutation affects *UCA1* expression in CRC tissue. Furthermore, the plasma *UCA1* levels in the present study may reflect the results obtained for *UCA1* expression in CRC tissue.

Detailed molecular subtypes, including the CpG island methylator phenotype, microsatellite instability, chromosomal instability, and p53, KRAS, or *BRAF* mutation, have recently been reported (3, 30), and many differences in cancer progression and genomic or molecular status have been elucidated between right- and left-sided CRC (2, 4). In the

Table III. *Clinicopathological features of patients with right-sided colorectal cancer according to plasma urothelial carcinoma-associated 1 (UCA1) level.*

Variable		n	Relative plasma UCA1 level, n (%)		*p*-Value
			≤0.158	>0.158	
Total	n (%)	40	22 (55.0)	18 (45.0)	
Age	≤70 Years	15 (37.5)	8 (20.0)	7 (17.5)	0.870
	>70 Years	25 (62.5)	14 (35.0)	11 (27.5)	
Gender	Male	16 (40.0)	8 (20.0)	8 (20.0)	0.604
	Female	24 (60.0)	14 (35.0)	10 (25.0)	
BRAF gene status	Wild-type	27 (67.5)	12 (30.0)	15 (37.5)	0.048
	Mutant	13 (32.5)	10 (25.0)	3 (7.5)	
Tumor size	≤40 mm	14 (35.0)	9 (22.5)	5 (12.5)	0.384
	>40 mm	26 (65.0)	13 (32.5)	13 (32.5)	
Histological type[a]	Tub	35 (87.5)	18 (45.0)	17 (42.5)	0.212
	Other	5 (12.5)	4 (10.0)	1 (2.5)	
Lymphatic invasion[a]	Negative	11 (27.5)	7 (17.5)	4 (10.0)	0.497
	Positive	29 (72.5)	15 (37.5)	14 (35.0)	
Venous invasion[a]	Negative	12 (30.0)	6 (15.0)	6 (15.0)	0.678
	Positive	28 (70.0)	16 (40.0)	12 (30.0)	
pT Factor[a]	1-3	27 (67.5)	12 (30.0)	15 (37.5)	0.048
	4	13 (32.5)	10 (25.0)	3 (7.5)	
pN Factor[a]	Negative	16 (40.0)	8 (20.0)	8 (20.0)	0.604
	Positive	24 (60.0)	14 (35.0)	10 (25.0)	
pStage[a]	1-3a	26 (65.0)	13 (32.5)	13 (32.5)	0.384
	3b-4	14 (35.0)	9 (22.5)	5 (12.5)	

BRAF: B-Raf proto-oncogene serine/threonine kinase; Tub: tubular adenocarcinoma. [a]According to the ninth edition of the Japanese Classification of Colorectal Carcinoma (17).

Table IV. *Clinicopathological features of patients with left-sided colorectal cancer according to plasma urothelial carcinoma-associated 1 (UCA1) level.*

Variable		n	Relative plasma UCA1 level, n (%)		*p*-Value
			≤0.158	>0.158	
Total	n (%)	36	16 (44.4)	20 (55.6)	
Age	≤70 Years	24 (66.7)	11 (30.6)	13 (36.1)	0.812
	>70 Years	12 (33.3)	5 (13.9)	7 (19.4)	
Sex	Male	18 (50.0)	12 (33.3)	6 (16.7)	0.006
	Female	18 (50.0)	4 (11.1)	14 (38.9)	
Tumor size	≤40 mm	20 (55.6)	8 (22.2)	12 (33.3)	0.549
	>40 mm	16 (44.4)	8 (22.2)	8 (22.2)	
Histological type[a]	Tub	35 (97.2)	16 (44.4)	19 (52.8)	0.274
	Other	1 (2.8)	0 (0)	1 (2.8)	
Lymphatic invasiona	Negative	11 (30.6)	7 (19.4)	4 (11.1)	0.149
	Positive	24 (66.7)	9 (25.0)	15 (41.7)	
Venous invasion[a]	Negative	10 (27.8)	6 (16.7)	4 (11.1)	0.283
	Positive	25 (69.4)	10 (27.8)	15 (41.7)	
pT Factor[a]	1-3	25 (69.4)	14 (38.9)	11 (30.6)	0.030
	4	11 (30.6)	2 (5.6)	9 (25.0)	
pN Factor[a]	Negative	10 (27.8)	6 (16.7)	4 (11.1)	0.245
	Positive	26 (72.2)	10 (27.8)	16 (44.4)	
pStage[a]	1-3a	21 (58.3)	13 (36.1)	8 (22.2)	0.011
	3b-4	15 (41.7)	3 (8.3)	12 (33.3)	

Tub: Tubular adenocarcinoma. [a]According to the ninth edition of the Japanese Classification of Colorectal Carcinoma (17).

present study, higher plasma *UCA1* levels were associated with advanced cases in left-sided CRC (Table IV). This result is consistent with previous findings which showed that higher *UCA1* expression in cancer tissue was associated with cancer progression and a poor prognosis (12, 13). On the other hand, in right-sided CRC, a lower plasma *UCA1* level was associated with more advanced cases, and correlated with *BRAF* gene mutation (Table III). These differences between left- and right-sided CRC may be influenced by the mutation status of the *BRAF* gene.

The frequency of *BRAF* mutation in CRC was previously reported to be approximately 5-10%, with the most common type of *BRAF*-activating mutation being a valine to glutamine acid change at codon 600 (*BRAF* V600E) (29, 31). This *BRAF* mutation up-regulates the mitogen-activated protein kinase signaling pathway and plays essential roles in cellular proliferation, apoptosis, and survival (29-31). The *BRAF* mutation is generally mutually exclusive from *KRAS* mutations, and is clinically related to cancer progression, chemoresistance, and a poor prognosis, particularly in metastatic CRC (31). Confirmation of the *BRAF* mutation status before treatment is important because it is closely involved in treatment selection due to chemoresistance. Previous studies showed that the *BRAF* mutation status in primary CRC and matched metastatic lesions were in good concordance (31, 32). However, the sample sizes of previous studies were very small and discrepancies were also reported (33). Moreover, the effects of previous chemotherapy on the mutation status need to be considered. Therefore, particularly in patients with recurrence, the prediction of the *BRAF* mutation status by plasma *UCA1* level would be useful as a liquid biopsy because tissue collection is often difficult in these patients.

In the present study, there are important issues that need to be resolved. The discrepancy between the lower plasma *UCA1* level in patients with CRC and higher *UCA1* expression in cancer tissue needs to be discussed. One important factor is *BRAF* gene mutation and reduced *UCA1* level in cancer tissue and plasma. However, Barbagallo *et al*. showed that the *UCA1* level in serum exosomes of patients with CRC was reduced compared with that of healthy controls, while the expression of *UCA1* in CRC biopsy tissue was up-regulated relative to that in non-cancerous tissue (14). Therefore, other mechanisms may suppress the plasma *UCA1* level. Furthermore, the reason why plasma and tissue *UCA1* levels were lower in patients with *BRAF* gene mutation remains unclear. *UCA1* expression is regulated by epigenetic and transcriptional factors, such as the direct binding of the transcription factor SP111 (34), or activation of the Hippo pathway (19). These factors need to be examined; however, we propose another possibility. Previous studies detected *BRAF* mutations in small polyps at early carcinogenesis (29, 30). In these tissues, the mitogen-activated protein kinase pathway will already be up-regulated by activating *BRAF* pathway, and, thus, *UCA1* may not need to be up-regulated. This mechanism will also lead to mutually exclusive relationships between KRAS and *BRAF*

Table V. *Patient background analyzed in tissue sample (n=26).*

Total	Value
Age (years)	
Median (range)	76 (52-94)
Gender	
Male	14
Female	12
Tumor location	
Right	18
Left	8
BRAF status of right-sided colon cancer	
Mutant	9
Wild-type	9
Macroscopic type, n[a]	
1	2
2	21
3	3
Tumor size, mm	
Median (range)	45 (23-90)
Histological type, n[a]	
Tub1	17
Tub2	6
Por	1
Sig	1
Muc	1
Lymphatic invasion, n[a]	
0	10
1	8
2	1
3	7
Venous invasion, n[a]	
0	8
1	15
2	2
3	1
pT Factor, n[a]	
1	0
2	1
3	19
4	6
pN Factor, n[a]	
0	13
1	8
2	5
pStage, n[a]	
1	0
2	13
3	13
4	0

BRAF: B-Raf proto-oncogene serine/threonine kinase; Left: left-sided colon and rectum; Muc: mucinous adenocarcinoma; Por: poorly differentiated adenocarcinoma; Right: right-sided colon; Sig: signet-ring cell carcinoma; Tub1: tubular adenocarcinoma well-differentiated type; Tub2: tubular adenocarcinoma moderately differentiated type. [a]According to the ninth edition of the Japanese Classification of Colorectal Carcinoma (17).

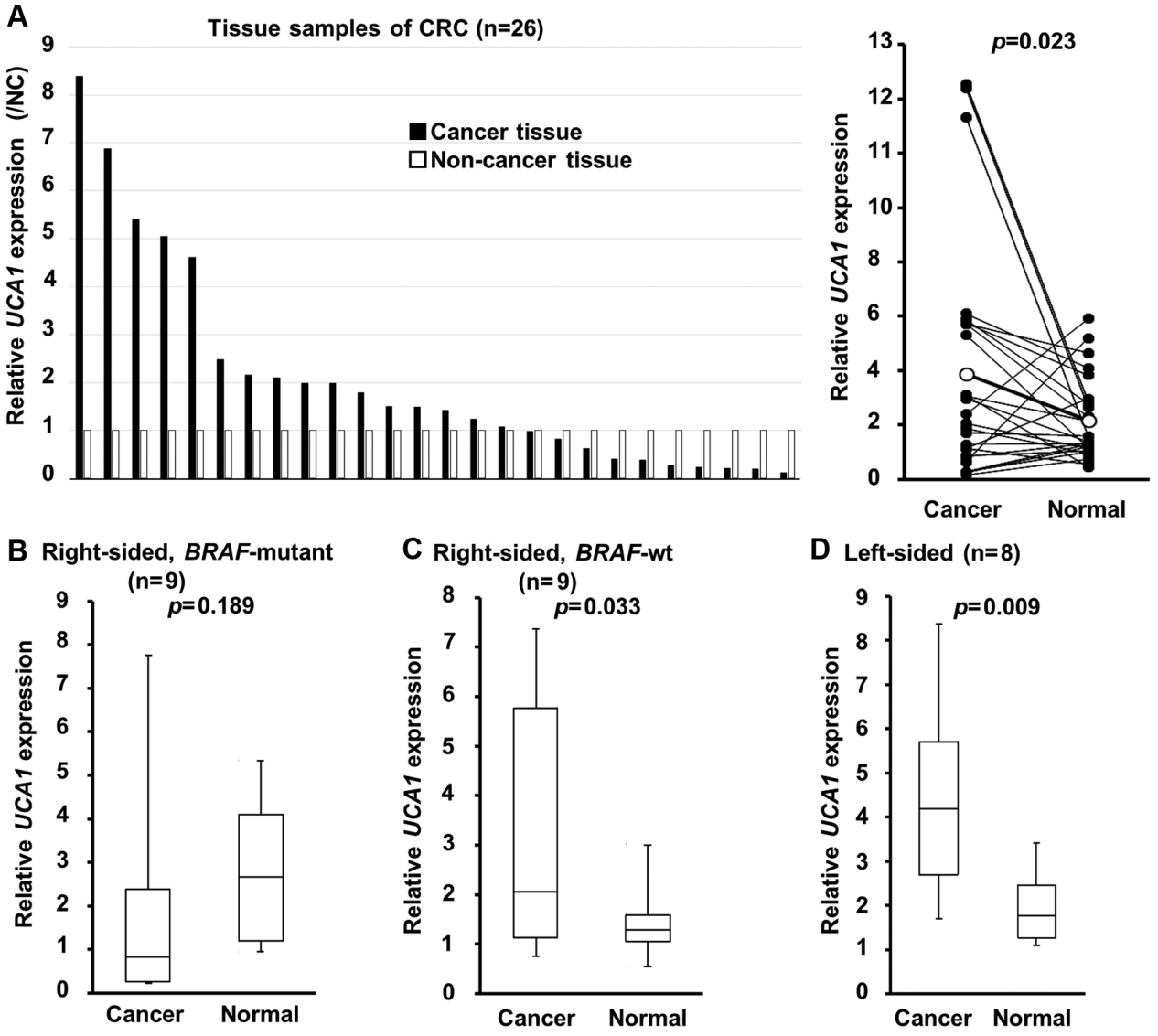

Figure 2. *Urothelial carcinoma associated 1 (UCA1) expression in colorectal cancer (CRC) tissue. A: Relative UCA1 expression in cancer and paired non-cancer tissue is shown. B-D: Relative UCA1 expression in cancer and paired non-cancer tissue was compared among patients with B-Raf proto-oncogene serine/threonine kinase (BRAF)-mutant right-sided CRC (B, n=9), BRAF-wild-type (wt) right-sided CRC (C, n=9), and left-sided CRC (D, n=8). Upper and lower limits of the box plots and the line inside the boxes indicate the 75th and 25th percentiles and the median, respectively.*

mutations. In the future, these molecular mechanisms need to be investigated in more detail.

There are also some limitations of the present study. The small patient population and limited sample holding status prevent more general conclusions from being reached. Therefore, examinations of large-scale samples are needed. In tissue and plasma analyses, the frequency of the *BRAF* mutation was increased because we focused on its effects on *UCA1* expression and selected samples with this mutation. Therefore, although it was not possible to examine clinicopathological features and prognosis in tissue samples according to *UCA1* expression, plasma analyses of right- and left-sided CRC in the present study was warranted.

In conclusion, the plasma *UCA1* level is a useful biomarker for the detection of patients with CRC and prediction of clinicopathological features. Moreover, it may predict *BRAF* gene mutation and facilitate treatment selection for patients with CRC.

Conflicts of Interest

The Authors declare no conflicts of interest for this study.

Authors' Contributions

Maeda T, Konishi H, and Otsuji E were involved in study design and data interpretation. Maeda T, Konishi H, Takaki W, Takabatake K, Matsubara D, Shoda K, Arita T, Shimizu H, Komatsu S, Shiozaki A, and Kuriu Y were involved in the sample collection and the data analysis. All Authors revised the article and approved the submission.

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