Original Article

Tandem Repeat Variation Near the *HIC1* (Hypermethylated in Cancer 1) Promoter Predicts Outcome of Oxaliplatin-Based Chemotherapy in Patients With Metastatic Colorectal Cancer

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BACKGROUND: The hypermethylated in cancer 1/sirtuin 1 (HIC1/SIRT1) axis plays an important role in regulating the nucleotide excision repair pathway, which is the main oxaliplatin-induced damage-repair system. On the basis of prior evidence that the variable number of tandem repeat (VNTR) sequence located near the promoter lesion of *HIC1* is associated with *HIC1* gene expression, the authors tested the hypothesis that this VNTR is associated with clinical outcome in patients with metastatic colorectal cancer who receive oxaliplatin-based chemotherapy. **METHODS:** Four independent cohorts were tested. Patients who received oxaliplatin-based chemotherapy served as the training cohort (n = 218), and those who received treatment without oxaliplatin served as the control cohort (n = 215). Two cohorts of patients who received oxaliplatin-based chemotherapy were used for validation studies (n = 176 and n = 73). The VNTR sequence near *HIC1* was analyzed by polymerase chain reaction analysis and gel electrophoresis and was tested for associations with the response rate, progression-free survival, and overall survival. **RESULTS:** In the training cohort, patients who harbored at least 5 tandem repeats (TRs) in both alleles had a significantly shorter PFS compared with those who had fewer than 4 TRs in at least 1 allele (9.5 vs 11.6 months; hazard ratio, 1.93; *P* = .012), and these findings remained statistically significant after multivariate analysis (hazard ratio, 2.00; 95% confidence interval, 1.13-3.54; *P* = .018). This preliminary association was confirmed in the validation cohort, and patients who had at least 5 TRs in both alleles had a worse PFS compared with the other cohort (7.9 vs 9.8 months; hazard ratio, 1.85; *P* = .044). **CONCLUSIONS:** The current findings suggest that the VNTR sequence near *HIC1* could be a predictive marker for oxaliplatin-based chemotherapy in patients with metastatic colorectal cancer. ***Cancer*** **2017;123:4506-14**. © *2017 American Cancer Society.*

KEYWORDS: hypermethylated in cancer 1 (HIC1), metastatic colorectal cancer, oxaliplatin, predictive marker, variable number of tandem repeat (VNTR) polymorphism.

INTRODUCTION

Acquired resistance to chemotherapy and molecularly targeted therapies used to treat human cancers is mediated by molecular alterations. Thus, a better understanding of these alterations could lead to the discovery and identification of potential biomarkers to predict treatment responses as well a reduction in acquired resistance to such therapies.

Oxaliplatin is a third-generation platinum drug that is used as a chemotherapy backbone to treat patients with metastatic colorectal cancer (mCRC) in combination with infused 5-fluorouracil/leucovorin (FOLFOX), capecitabine (CapeOX), and infused 5-fluorouracil/leucovorin plus irinotecan (FOLFOXIRI).[1] The antitumor effect of oxaliplatin is considered to be the disruption of DNA replication and transcription, primarily by cross-linking the same strand of DNA.[2] Oxaliplatin-induced DNA damage is primarily repaired by the nucleotide excision repair (NER) pathway, and activated NER may contribute to oxaliplatin resistance.[3] Therefore, genes involved in the NER pathway, such as ERCC1 (excision repair cross-complementation group 1), have been extensively studied over the past decade as predictive markers for both oxaliplatin efficacy and resistance.[4-6] However, no predictive markers for oxaliplatin have been prospectively validated.

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The hypermethylated in cancer 1/sirtuin 1 (HIC1/SIRT1) axis plays an important role in the regulation of DNA repair pathways.[7] SIRT1 is a deacetylase that regulates several types of substrates or proteins to maintain genomic instability. Among the targets of SIRT1, xeroderma pigmentosum complementation group A (XPA), which is involved in the NER pathway, is deacetylated by SIRT1; this modification leads to the promotion of the NER pathway[8] (Fig. 1). HIC1, a sequence-specific transcriptional repressor, directly binds to the SIRT1 promoter and suppresses SIRT1 transcription.[9] Thus, the inactivation of HIC1 results in the upregulation of SIRT1 expression and activates the NER pathway, which may contribute to oxaliplatin resistance.

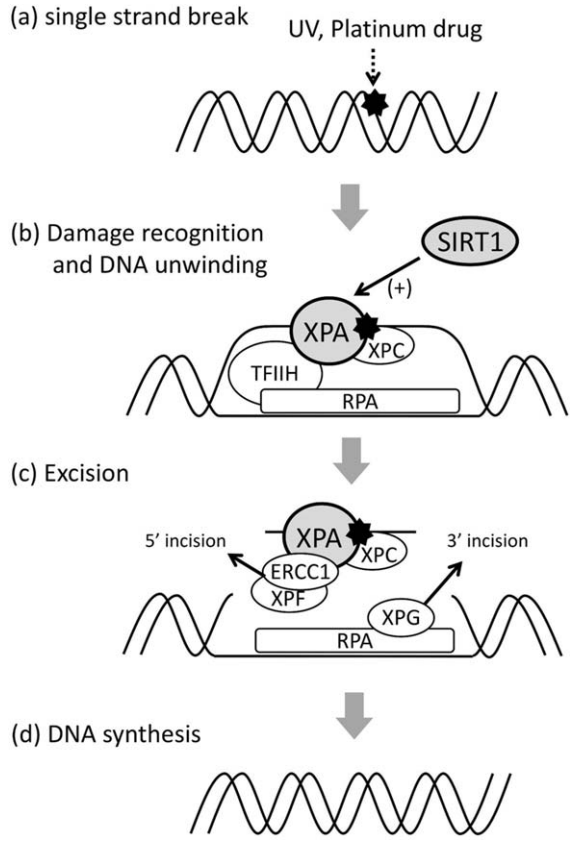

Figure 1. The nucleotide excision repair pathway is illustrated. (a) A single strand break induced by ultraviolet light (UV) or platinum drugs is recognized by damage-sensor molecules, such as xeroderma pigmentosum complementation group C (XPC), followed by the binding of several other proteins, such as transcription factor II (TFIIH), XPA, and replication protein A (RPA). (b) TFIIH unwinds the DNA duplex, whereas XPA verifies the DNA damage by binding to the chemically altered nucleotides. (c) Then, XPA allows for the binding of a complex of nucleases (excision repair cross-complementation group 1 [ERCC1]/XPF), which is directed to the damaged strand by RPA to create an incision 5′ to the lesion. After the 5′ incision, RPA recruits a nuclease (XPG), which makes an incision on the 3′ side of the damaged lesion. (d) After this process, DNA polymerase synthesizes new DNA to replace the damage. XPA is deacetylated by sirtuin 1 (SIRT1), leading to nucleotide excision repair promotion by facilitating its interaction with RPA.

Given prior evidence that the variable number of tandem repeat (VNTR) sequence located near the *HIC1* promoter, denoted as D17S5, is associated with *HIC1* gene expression (Supporting Fig. 1; see online supporting information), we hypothesized that the VNTR near *HIC1* may cause interindividual differences in clinical outcome in patients with mCRC who receive oxaliplatin-based chemotherapy. This study presents the potential value of the VNTR sequence near *HIC1* as a polymorphic genetic marker in predicting oxaliplatin efficacy in different patient cohorts.

MATERIALS AND METHODS

Patients and Samples

This study consists of 4 independent cohorts: a control cohort, a training cohort, and 2 validation cohorts. Patients in each cohort are individuals with mCRC who were enrolled in clinical trials and received chemotherapy with or without oxaliplatin (Table 1). Of the patients enrolled in the randomized phase 3 Combination Chemotherapy and Bevacizumab as First-Line Therapy in Treating Patients With Metastatic Colorectal Cancer (TRIBE) study,[10] those who received oxaliplatin-based chemotherapy (FOLFOXIRI + bevacizumab) served as the training set (TRIBE-B cohort; n = 218), and those who received treatment without oxaliplatin (FOLFIRI + bevacizumab) served as the control set (TRIBE-A cohort; n = 215). Two cohorts of patients receiving oxaliplatin-based chemotherapy were used for validation studies (the Maintenance Bevacizumab Only or Bevacizumab Plus Metronomic Chemotherapy in Advanced Colorectal Cancer [MOMA] cohort; n = 176; USA cohort, n = 73). All patients provided informed consent before entering the randomized trials along with information regarding the use of their tumor tissue to explore relevant molecular parameters. This study was conducted in accordance with the Reporting Recommendations for Tumor Marker Prognostic Studies (REMARK). The tissue analysis was approved by the University of Southern California Institutional Review Board of Medical Sciences and was conducted at the University of Southern California/Norris Comprehensive Cancer Center in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines.

TABLE 1. Summary of the Cohorts Used in This Study

Variable	Control Cohort TRIBE-A	Training Cohort TRIBE-B	Validation Cohort MOMA	Validation Cohort USA
Clinical trial	TRIBE study (arm A) randomized phase 3 (2008-2011, Italy)	TRIBE study (arm B) randomized phase 3 (2008-2011, Italy)	MOMA study randomized phase 2 (2012-2016, Italy)[a]	3C-04-10 Study phase 2 (2005-2009, USA)[b]
Ethnic background	Caucasian	Caucasian	Caucasian	Caucasian, 32.9%; Hispanic, 35.6%; Asian 26%; African American, 5.5%
No. of patients[c]	215	218	176	73
Chemotherapy	FOLFIRI + bevacizumab[d]	FOLFOXIRI + bevacizumab	FOLFOXIRI + bevacizumab	FOLFOX or CapeOx + bevacizumab
Oxaliplatin-based	No	Yes	Yes	Yes

Abbreviations: CapeOx, capecitabine and oxaliplatin; FOLFOX, infused 5-fluorouracil/leucovorin and oxaliplatin; FOLFOXIRI, infused 5-fluorouracil/leucovorin plus irinotecan and oxaliplatin; MOMA, Maintenance Bevacizumab Only or Bevacizumab Plus Metronomic Chemotherapy in Advanced Colorectal Cancer; TRIBE, Combination Chemotherapy and Bevacizumab as First-Line Therapy in Treating Patients With Metastatic Colorectal Cancer.
[a] Clinicaltrials.gov identifier: NCT02271464.
[b] Clinicaltrials.gov identifier: NCT00159432.
[c] Patients without available samples were excluded from this study.

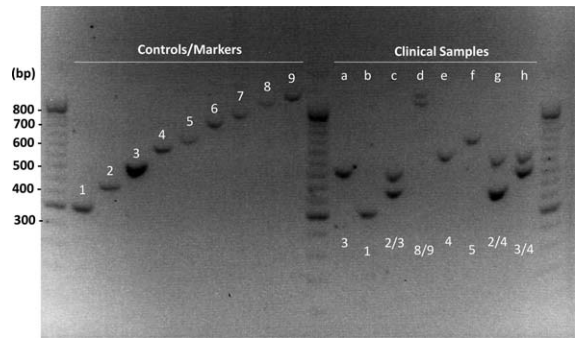

Figure 2. Genotype identification of the variable number of tandem repeat marker near hypermethylated in cancer (*HIC*). The size of the polymerase chain reaction product size was calculated (in base pairs [bp]) by the following formula: 260 + 70 × N, where *N* indicates the number of tandem repeats [TRs]). Control samples that had from 1 to 9 TRs were used as markers to identify the genotype for clinical samples. For example, (a) the first sample depicts a genotype of homozygous 3/3 TRs, and (d) another sample shows a genotype of heterozygous 8/9 TRs.

Genotyping of the VNTR Near HIC1

Tandem repeats (TRs) at the D17S5 loci are positioned approximately 1000 base pairs (bp) upstream of the HIC1 transcription start site, and a 70-bp-long sequence is tandemly repeated from once to greater than 10 times.[11] Genomic DNA was extracted from the blood using the QIAamp DNAeasy Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. To determine the genotypes for TR length, a DNA fragment containing the TR region was amplified by polymerase chain reaction (PCR) using a forward primer (5′-GTTGGGAG CGGGGAAATGCA-3′) and a reverse primer (5′-TGCATTGCTAACCTGCCCCA-3′). The PCR protocol is consisted of: 1 cycle for 5 minutes at 95°C; 30 cycles for 30 seconds at 95°C, 30 seconds at 60°C, and 90 seconds at 72°C; and 1 cycle for 7 minutes at 72°C. PCR products were separated on 1.2% agarose gels containing biotin and were detected by ultraviolet fluorescence (Fig. 2). Investigators involved in VNTR analyses were blinded to patient clinical data.

Statistical Analysis

To explore the predictive value of the VNTR sequence near *HIC1* for oxaliplatin-based chemotherapy, the primary outcome measure was defined as progression-free survival (PFS), which was calculated from the start date of first-line chemotherapy until the first observation of disease progression or death. If progression or death was not observed, then PFS was the day of the last computed tomography scan. We also evaluated the association with response rate (RR) and overall survival (OS), which are defined as the percentage of patients experiencing complete responses and partial responses, according to Response Evaluation Criteria in Solid Tumors (RECIST) guidelines, and the period from the start date of therapy to the date of death or of last contact if the patient remained alive, respectively. Differences in baseline patient characteristics between the 4 cohorts were examined using the Fisher exact test or the Kruskal-Wallis test, as appropriate. The power to detect an association between a VNTR in the recessive model and PFS was 80% when the minimum hazard ratio (HR) varied from 1.84 to 3.19 in 20% to 40% of patients with 2 copies of the

TABLE 2. Baseline Clinical Characteristics of the TRIBE-A, TRIBE-B, MOMA, and USA Cohorts

Characteristic	No. of Patients (%)				P[a]
	TRIBE-A, n = 215	TRIBE-B, n = 218	MOMA, n = 176	USA, n = 73	
Sex					
Men	128 (59.5)	130 (59.6)	100 (56.8)	45 (61.6)	.89
Women	87 (40.5)	88 (40.4)	76 (43.2)	28 (38.4)	
Age, y					
Median [range]	60 [29-75]	60 [29-75]	61 [23-74]	55 [28-74]	.002
≤65	155 (72.1)	150 (68.8)	125 (71)	61 (83.6)	.11
>65	60 (27.9)	68 (31.2)	51 (29)	12 (16.4)	
Performance status					
ECOG 0	177 (82.3)	195 (89.4)	153 (86.9)	—	.12
ECOG 1	37 (17.2)	23 (10.6)	23 (13.1)	—	
Unknown[b]	1 (0.5)				
Primary tumor site					
Right	53 (24.7)	73 (33.5)	63 (35.8)	19 (26)	.11
Left	147 (68.4)	134 (61.5)	113 (64.2)	53 (72.6)	
Unknown[b]	15 (7)	11 (5)		1 (1.3)	
Time to metastasis					
Synchronous	177 (82.3)	172 (78.9)	—	62 (84.9)	.45
Metachronous	38 (17.7)	46 (21.1)	—	11 (15.1)	
Liver metastasis					
Yes	66 (30.7)	76 (34.9)	52 (29.5)	33 (45.2)	.083
No	149 (69.3)	142 (65.1)	124 (70.5)	40 (54.8)	
No. of metastatic sites					
1	92 (42.8)	96 (44)	—	41 (56.2)	.12
≥2	123 (57.2)	122 (56)	—	32 (43.8)	
Adjuvant chemotherapy					
Yes	26 (12.1)	27 (12.4)	—	4 (5.5)	.24
No	189 (87.9)	191 (87.6)	—	69 (94.5)	
RAS wild type					
Yes	53 (24.7)	58 (26.6)	43 (24.4)	—	.24
No	110 (51.2)	108 (49.5)	119 (67.6)	—	
Unknown[b]	52 (24.2)	52 (23.9)	14 (8)	—	

Abbreviations: ECOG, Eastern Cooperative Oncology Group; MOMA, Maintenance Bevacizumab Only or Bevacizumab Plus Metronomic Chemotherapy in Advanced Colorectal Cancer; *RAS*, viral rat sarcoma gene; TRIBE, Combination Chemotherapy and Bevacizumab as First-Line Therapy in Treating Patients With Metastatic Colorectal Cancer.

[a] *P* values were based on the chi-square test or the Kruskal-Wallis test, as appropriate

[b] These individuals were not included in the analysis.

minor allele using a 2-sided log-rank test at a significance level of .05 in the training cohort (n = 218; 155 PFS events). The power was greater than 70% and 46%, respectively, using the same test to detect the same range of HRs with the same allele frequencies in the recessive model in the validation cohorts (MOMA cohort, n = 176, 119 PFS events; USA cohort, n = 73, 62 PFS events). The associations between polymorphisms and PFS and OS were investigated using Kaplan-Meier curves, the log-rank test, and the Fisher exact test. A Cox proportional-hazards regression model with stratification factors was fitted to re-evaluate the association between the VNTR and PFS and OS, considering imbalances in the distributions of baseline characteristics among cohorts. The baseline demographics and clinical characteristics that remained significantly associated with endpoints after the multivariate analysis ($P < .1$) were included in the final model. All analyses were performed with 2-sided tests at a significance level of .05 using SAS (version 9.4; SAS Institute, Cary, NC,).

RESULTS

Compared with the TRIBE-B training cohort, no differences in characteristics were observed in the TRIBE-A control and MOMA validation cohorts. The USA validation cohort was composed of younger patients (Table 2). The median PFS, OS, and follow-up was 9.7, 25.6, and 49.9 months, respectively, in the TRIBE-A cohort; 11.4, 28.6, and 48.0 months, respectively, in the TRIBE-B cohort; 9.5, 25.4, and 25.3 months, respectively, in the MOMA cohort; and 13.0, 31.1, and 43.3 months, respectively, in the USA cohort.

On the basis of our previous findings that the VNTR sequence near *HIC1* has different expression patterns for *HIC1* between fewer than 4 TRs and at least 5 TRs (Supporting Fig. 1; see online supporting

information), we applied a cutoff value for the number of TRs between 4 and 5. Alleles with fewer than 4 TRs and those with at least 5 TRs were defined as small (S) and large (L), respectively. We compared clinical outcomes between patients who had <4 TRs in both alleles (S/S), those who had <4 TRs in 1 allele (S/L), and those who had ≥5 TRs in both alleles (L/L).

Association of TRs With Clinical Outcomes in the Training and Control Cohorts

Table 3 details the association between the VNTR near *HIC1* and clinical outcomes. The frequency of genotypes S/S, S/L, and L/L were 77%, 13%, and 10%, respectively, in the TRIBE-A cohort, and 82%, 9%, and 9%, respectively, in the TRIBE-B cohort. In the TRIBE-B training cohort, patients who had the L/L genotype had a significantly shorter PFS compared with those who had either the S/L or the L/L genotype. The median PFS was 9.5 months for patients with the L/L genotype compared with 11.6 months for those with the other genotypes (HR, 1.93; 95% confidence interval [CI], 1.11-3.35; $P = .012$) (Fig. 3B). This difference remained statistically significant after multivariate analysis (HR, 2.00; 95% CI, 1.13-3.54; $P = .018$). Conversely, these correlations were not observed in the TRIBE-A control cohort (Fig. 3A). These data suggest that the VNTR sequence near *HIC1* may be associated with oxaliplatin efficacy in patients with mCRC.

Validation Studies

In the *MOMA* validation cohort (S/S genotype, 81%, S/L genotype, 14%, L/L genotype, 5%), the preliminary association observed in the training cohort was confirmed. Patients who had the L/L genotype had a significantly worse PFS compared with those who had either the S/L or S/S genotype after univariate analysis (median PFS, 7.9 vs 9.8 months; HR, 2.13; $P = .044$) (Fig. 3C), yet this correlation was not statistically significant after multivariate analysis (HR, 1.92; 95% CI, 0.87-4.24; $P = .105$).

Furthermore, to exclude the impact of irinotecan on clinical outcomes, we examined the association of the VNTR sequence in patients who received treatment with FOLFOX or CapeOx backbone in the USA cohort. There was no evidence of an association with PFS between patients with the L/L genotype and those with the S/L or S/S genotype (Fig. 3D). However, patients who had more than 5 TRs in both alleles (n = 4) had a borderline significant trend toward shorter PFS compared with the other cohort (n = 69) after univariate analysis (8.1 vs 13.0 months; HR, 2.28; 95% CI, 0.80-6.47; $P = .098$) (Supporting Fig. 2; see online supporting information).

Subgroup Analysis

Given the previously reported biologic features of CRC,[12-14] subgroup analyses by sex, primary tumor location, and viral rat sarcoma gene (*RAS*) status were performed. Primary tumor locations were grouped into those on the right side (cecum, ascending colon, and transverse colon) and those on the left side (descending colon, sigmoid colon, and rectum). *RAS* status was divided into 2 groups: all *RAS* wild-type and other types. Figure 4 illustrates the associations of the VNTR sequence near *HIC1* with PFS in each subgroup of the TRIBE-B training cohort. Significant associations were observed, particularly in patients with mCRC who had tumors derived from the left side (univariate analysis: PFS, 8.6 vs 12.4 months; HR, 2.68; $P = .003$; multivariate analysis: HR, 2.92; 95% CI, 1.38-6.15; $P = .005$) or with *RAS* wild-type tumors (univariate analysis: PFS, 9.3 vs 11.3 months; HR, 2.78; $P = .042$; multivariate analysis: HR, 5.51; 95% CI, 1.49-20.4; $P = .011$) after both univariate and multivariate analyses. However, these associations were not confirmed in the MOMA validation cohort (data not shown).

DISCUSSION

Although many drug combinations have emerged as possible options as first-line chemotherapy for patients with mCRC, several controversial issues remain, including the choice of an optimal chemotherapy backbone. In the clinic, either FOLFIRI or FOLFOX (or CapeOx) is selected as the first-line chemotherapy backbone based on practical issues, patient preference, and toxicity profiles, because no substantial differences in efficacy have been demonstrated between these regimens.[15,16] FOLFOXIRI has been proposed as a feasible option with a high antitumor activity and acceptable toxicity,[17,18] yet the eligibility criteria have some limitations because of the high intensity of the treatment. Therefore, biomarkers predicting drug efficacy, resistance, and/or toxicity could help with selecting the most favorable first-line chemotherapy regimen for patients with mCRC.

In the current study, we observed that the VNTR sequence near *HIC1* was significantly associated with PFS in patients who received oxaliplatin-based chemotherapy, but not in those who received irinotecan-based chemotherapy, suggesting that this VNTR may be a predictive marker for oxaliplatin efficacy. TRs, which are stretches of DNA composed of 2 or more contiguous copies of the

TABLE 3. Association of the Variable Number of Tandem Repeat Polymorphism Near *HIC1* With Clinical Outcome in the 4 Cohorts

				PFS					OS				
	RR			Univariate Analysis[a]			Multivariate Analysis[b]		Univariate Analysis[a]			Multivariate Analysis[b]	
Genotype[c]	No.	%	*P*	Median (95% CI), mo	HR (95% CI)	*P*	HR (95% CI)	*P*	Median (95% CI), mo	HR (95% CI)	*P*	HR (95% CI)	*P*
Training cohort: TRIBE-B													
S/S	179	68	.58	11.7 (10.4-13.1)	1.00 (Ref)	.041[d]	1,00 (Ref)	.049[d]	28.5 (25.0-34.2)	1.00 (Ref)	.61	1.00 (Ref)	.78
S/L	19	76		11.0 (8.9-14.0)	1.11 (0.63-1.96)		0.80 (0.41-1.56)		25.8 (10.4-51.8)	1.16 (0.67-2.02)		0.94 (0.50-1.76)	
L/L	20	61		9.5 (3.7-13.2)	1.95 (1.12-3.39)		1.96 (1.10-3.48)		30.8 (8.4-42.0)	1.27 (0.75-2.13)		1.19 (0.70-2.03)	
S/S + S/L	198	69	.60	11.6 (10.4-13.0)	1 (Ref)	.012[d]	1.00 (Ref)	.018[d]	28.5 (25.0-34.2)	1.00 (Ref)	.40	1.00 (Ref)	.50
L/L	20	61		9.5 (3.7-13.2)	1.93 (1.11-3.35)		2.00 (1.13-3.54)		30.8 (8.4-42.0)	1.25 (0.74-2.10)		1.20 (0.71-2.04)	
Validation cohort 1: MOMA													
S/S + S/L	167	65	.99	9.8 (8.8-10.4)	1 (Ref)	.044[d]	1.00 (Ref)	.105	25.4 (19.8-29.1)	1.00 (Ref)	.104	1.00 (Ref)	.217
L/L	9	67		7.9 (1.9-9.1)	2.13 (0.96-4.71)		1.92 (0.87-4.24)		14.4 (6.9-41.2)	2.07 (0.83-5.17)		1.79 (0.71-4.48)	
Validation cohort 2: USA													
S/S + S/L	66	60	.44	13.0 (10.1-15.8)	1 (Ref)	.52	1 (Ref)	.68	31.1 (24.2-42.7)	1.00 (Ref)	.70	1.00 (Ref)	.71
L/L	7	43		9.6 (7.0-20.7)	1.29 (0.59-2.84)		0.83 (0.34-2.02)		32.3 (9.8-37.7)	1.22 (0.43-3.45)		1.23 (0.42-3.61)	
Control cohort: TRIBE-A													
S/S + S/L	193	56	.24	9.7 (9.0-11.0)	1 (Ref)	.484	1.00 (Ref)	.079	25.6 (21.5-29.1)	1.00 (Ref)	.851	1.00 (Ref)	.240
L/L	22	71		9.5 (7.5-12.2)	1.19 (0.72-1.98)		1.61 (0.95-2.73)		25.5 (15.1-48.4)	0.95 (0.57-1.59)		1.38 (0.81-2.34)	

Abbreviations: CI, confidence interval; HR, hazard ratio; MOMA, Maintenance Bevacizumab Only or Bevacizumab Plus Metronomic Chemotherapy in Advanced Colorectal Cancer; OS, overall survival; PFS, progression-free survival; Ref, reference category; RR, response rate; TRIBE, Combination Chemotherapy and Bevacizumab as First-Line Therapy in Treating Patients With Metastatic Colorectal Cancer.

[a] *P* values were based on the Fisher exact test for tumor response, the log-rank test for PFS and OS in the univariate analysis.

[b] *P* values were based on the Wald test for PFS and OS in the multivariable Cox regression model and were adjusted for age, Eastern Cooperative Oncology Group (ECOG) performance status, primary tumor site, the number of metastatic sites, resection of the primary tumors, viral rat sarcoma gene (*RAS*) mutation status, and adjuvant chemotherapy in the TRIBE-A and TRIBE-B cohorts; adjusted for ECOG performance status and liver-limited metastasis in the MOMA cohort; and adjusted for the number of metastasis, treatment type, and sex in the USA cohort.

[c] Alleles with fewer than 4 tandem repeats (TRs) and those with at least 5 TRs were defined as small (S) and large (L), respectively; and clinical outcomes were compared between patients who had <4 TRs in both alleles (S/S genotype), those who had <4 TRs in 1 allele (S/L genotype), and those who had ≥5 TRs in both alleles (L/L genotype).

[d] $P < .05$ (correlation).

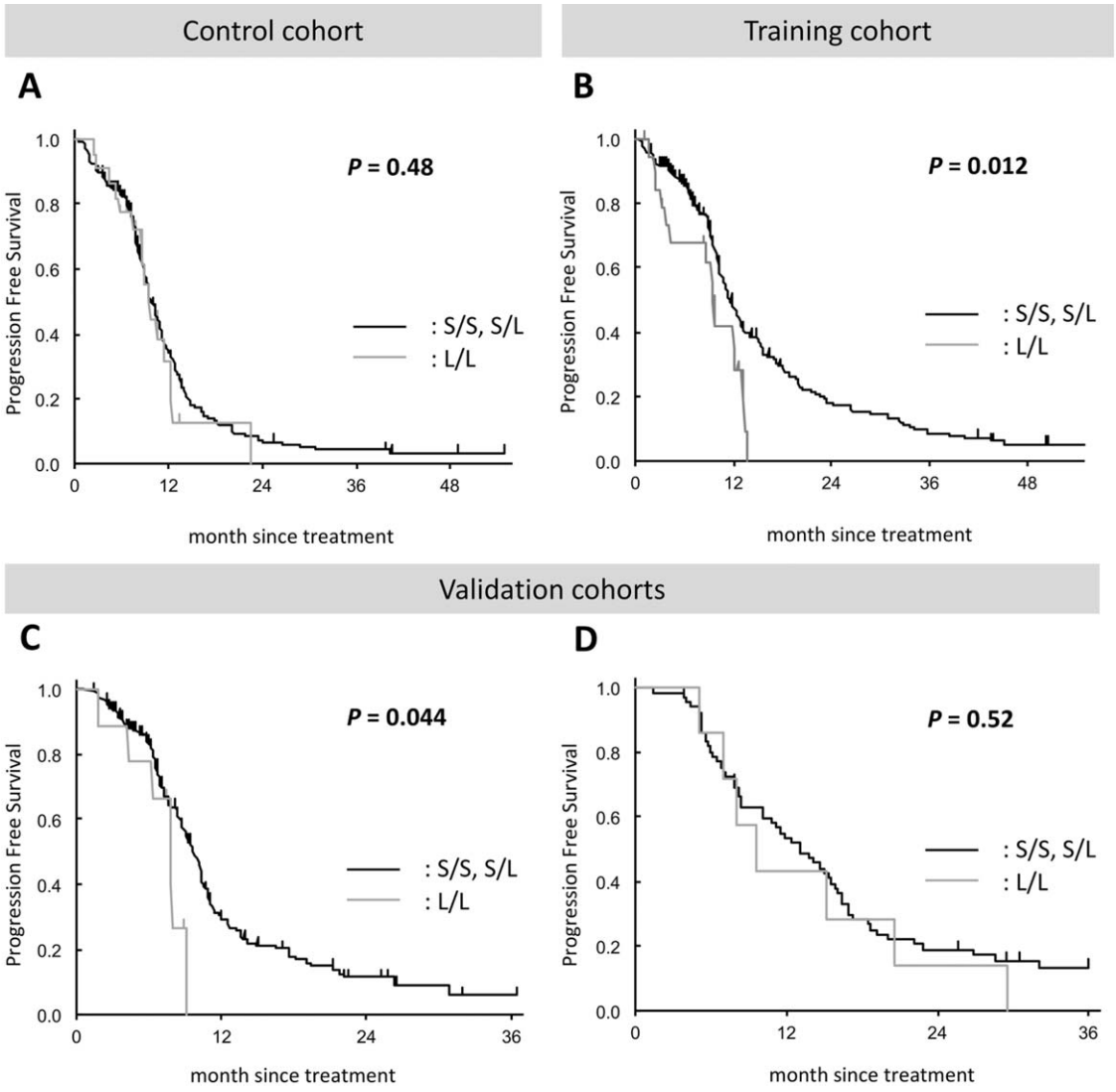

Figure 3. (A-D) Progression-free survival is illustrated in the 4 cohorts. Kaplan-Meier estimates of progression-free survival are illustrated according the number of tandem repeats (TRs), where alleles with fewer than 4 TRs and those with at least 5 TRs were defined as small (S) and large (L), respectively. S/S indicates 2 short alleles; L/L, 2 long alleles; S/L, 1 short and 1 long allele.

same genomic sequence, exhibit a length polymorphism that potentially could be used as a genetic marker. Although the functional role of TRs is unclear, growing evidence has revealed that certain TRs located in the 5′-untranslated region exert functional effects on gene expression by modulating the binding of transcription factors, the distance between promoter elements, splicing efficiency, or DNA structure.[19] We previously reported that the VNTR sequence near *HIC1* was significantly associated with HIC1 expression: Once the smaller allele exceeds 4 repeats, higher levels of HIC1 expression were no longer observed, and a trend of decreasing expression was evident (Supporting Fig. 1; see online supporting information). In this study, we observed a clear difference in PFS between patients who had fewer than 4 TRs and those who had at least 5 TRs. Although this observation was not confirmed in the USA cohort, potentially because of the small sample size, the observation that patients who had at least 6 TRs in both alleles had a trend toward worse PFS is consistent with the inverse correlation between larger numbers of TRs and lower levels of gene expression.

Downregulation of HIC1 associated with large numbers of TRs may lead to the overexpression of SIRT1 because of transcriptional suppression by HIC1.[9] The biologic rationale between HIC1/SIRT1 expression and

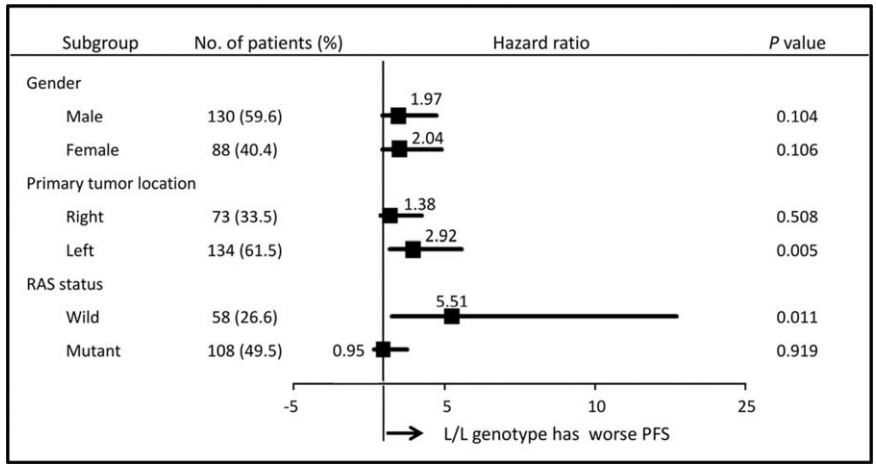

Figure 4. A subgroup analysis of progression-free survival (PFS) in the TRIBE-B cohort is illustrated. *P* values were based on the Wald test for progression-free survival in the multivariable Cox regression model and adjusted for age, Eastern Cooperative Oncology Group performance status, primary tumor site, number of metastatic sites, resection of the primary tumor, *RAS* mutation status, and adjuvant chemotherapy. L/L indicates the genotype with the presence of ≥5 tandem repeats in both alleles.

oxaliplatin efficacy, however, currently remains unclear. NER is a repair pathway for single-strand breaks: DNA damage induced by UV light and platinum drugs (eg, cisplatin, carboplatin, and oxaliplatin) is repaired by the NER system.[20-22] In the NER pathway, it is believed that XPA, a target of SIRT1, is the core factor responsible for platinum drug resistance,[8] because several studies have demonstrated that XPA is overexpressed in cisplatin-resistant cancers.[23-25] In the multistep process of DNA repair, XPA plays a role during the damage recognition or verification phase, interacting with RPA (replication protein A) (Fig. 1). Although the acetylated status of XPA reduces NER activity, deacetylation of XPA by SIRT1 could promote NER activity by facilitating its interaction with RPA, in turn leading to platinum drug resistance.[8] In a recent study by Asaka and colleagues,[26] a cell line that overexpressed SIRT1 exhibited higher viability after treatment with cisplatin than a control cell line. In a mouse xenograft model, tumors that overexpressed SIRT1 exhibited stronger resistance to cisplatin compared with control tumors. Taken together, these reports indicate that HIC1/SIRT1 expression levels affect NER activity through XPA modulation, resulting in interindividual differences in oxaliplatin efficacy.

In patients with mCRC, distinct molecular profiles based on sex, primary tumor site, and *RAS* status have been reported.[12-14] DNA repair systems may differ by primary tumor location, as represented by differences in microsatellite instability.[27] In addition, *RAS* status reportedly influences DNA damage responses.[28] A previous study identified a distinct ERCC1 expression profile based on primary tumor location and *RAS* status, suggesting the existence of regulatory mechanisms between the NER pathway, tumor location, and *RAS* status.[29] It is noteworthy that we observed herein a strong association between the VNTR polymorphism near *HIC1* and PFS, particularly in patients who had metastatic tumors derived from the left side of the colon or with *RAS* wild-type tumors. However, these associations were not confirmed in the validation study, potentially because of the small numbers of patients who had the minor L/L genotype. Future studies with a larger sample size are warranted to confirm these associations and underlying biologic mechanisms.

In conclusion, this study demonstrates for the first time the potential value of the VNTR polymorphism near *HIC1* as a predictive marker of outcome after oxaliplatin-based chemotherapy in patients with mCRC. If our findings are prospectively validated, then, because the TR polymorphism can be easily examined by PCR of a blood sample, the VNTR sequence near *HIC1* would be a promising biomarker to individualize the use of oxaliplatin in the clinic.

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CONFLICT OF INTEREST DISCLOSURES

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AUTHOR CONTRIBUTIONS

Satoshi Okazaki: Conceptualization, methodology, validation, investigation, and writing–original draft. **Marta Schirripa:** Investigation. **Fotios Loupakis:** Resources. **Shu Cao:** Formal analysis. **Wu Zhang:** Methodology. **Dongyun Yang:** Formal analysis. **Yan Ning:** Methodology. **Martin D. Berger:** Investigation. **Yuji Miyamoto:** Investigation. **Mitsukuni Suenaga:** Investigation. **Syma Iqubal:** Resources. **Afsaneh Barzi:** Resources. **Chiara Cremolini:** Resources. **Alfredo Falcone:** Resources. **Francesca Battaglin:** Resources. **Lisa Salvatore:** Resources. **Beatrice Borelli:** Resources. **Timothy G. Helentjaris:** Conceptualization. **Heinz-Josef Lenz:** Conceptualization, writing–review and editing, supervision, and funding acquisition.

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