Upregulation of WEE1 is a potential prognostic biomarker for patients with colorectal cancer

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Abstract. WEE1 is a serine/threonine protein kinase that inactivates cell division cycle 2 and is therefore a critical cell cycle regulator. Increased WEE1 expression has been observed in numerous types of human malignancies, including hepatocellular carcinoma and melanoma. WEE1 inhibition also results in evident anti-tumor effects in several human tumor cells including colon cancer cells, suggesting WEE1 as a potential therapeutic target for the treatment of cancer. However, the expression pattern of WEE1 in colorectal cancer (CRC) remains unclear. In the present study, WEE1 mRNA expression in 43 cases of CRC tissues matched with adjacent normal tissues was determined by reverse-transcription quantitative polymerase chain reaction. The results demonstrated that WEE1 mRNA expression was significantly increased in CRC tissues and that this upregulation correlated significantly with hepatic metastasis, distant metastasis and high tumor node metastasis (TNM) stage of CRC. Additionally, WEE1 protein in 102 CRC tissue samples was detected by immunohistochemistry, and positive staining of WEE1 was identified in more than half of patients with CRC. WEE1 staining scores were also observed to be associated with distant metastasis and high TNM stage of CRC. In addition, patients with CRC with high WEE1 staining score (2+ or 3+) exhibited either poorer overall survival or poorer disease-free survival compared with those with low WEE1 staining score (0 or 1+). The multivariable Cox model also identified a high WEE1 staining score as well as high TNM stage to be independent prognostic factors for CRC. In conclusion, WEE1 upregulation is associated with a high degree of malignancy and poor prognosis of CRC, suggesting WEE1 as a potential prognostic biomarker for CRC.

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Introduction

Colorectal carcinoma (CRC) is the third most common type of cancer worldwide and has increasing rates of incidence in the Asia-Pacific region, including China (1-3). A number of studies have shown that a variety of genetic and epigenetic alterations in both oncogenes and tumor suppressors are involved in the pathogenesis of CRC. The activation of oncogenes such as the ras gene and the inactivation of tumor suppressors such as adenomatous polyposis coli and p53 genes have been well documented in CRC (4-6). In addition, we previously identified certain genetic changes, including the downregulation of MUS81 structure-specific endonuclease subunit and epidermal growth factor-like protein 8 precursor, to be associated with this malignancy (7,8). However, further investigations are still necessary to clarify the tumorigenic pathway of CRC (9).

WEE1 is a member of the serine/threonine protein kinase gene family originally defined by Thuriaux *et al* (10) in fission yeast *Schizosaccharomyces pombe*. In mammals, WEE1 encodes a 94 kDa protein with 647 amino acid residues and composes a small gene family with myelin transcription factor 1 (11,12). WEE1 is located predominantly in the nucleus and selectively phosphorylates the phospho-cdc2 residue of the cell division cycle 2 and inactivates it (13,14). WEE1 is therefore a critical G2 checkpoint regulator that induces interphase and prevents the initiation of mitosis (15). Previous studies have shown that WEE1 expression is increased in various types of human malignancies including melanoma and vulvar squamous cell, ovarian and hepatocellular carcinoma (HCC) (16-19). Elevated WEE1 expression is also documented in glioblastoma and breast cancer cells, and the inhibition of WEE1 in these cells results in suppressed cellular proliferation and increased apoptosis (20-22). In addition, a potent and selective inhibitor of WEE1 protein, AZD1775, has demonstrated the ability to sensitize T cell acute lymphoblastic leukemia to cytarabine by promoting apoptosis over DNA repair (23). AZD1775, formerly termed MK-1775, may also enhance the therapeutic effects of chemotherapy agents, including 5-fluorouracil, doxorubicin, camptothecin and mitomycin C, in various p53-deficient colon cancer cells (24). Although these previous studies have suggested WEE1 as a promising target

in the therapy of human malignancies including CRC (25), the data regarding the expression pattern of WEE1 in human CRC tissues remains limited (26).

The present study therefore detected the expression levels of the WEE1 gene and protein in CRC tissues by reverse-transcription quantitative polymerase chain reaction (RT-qPCR) and immunohistochemistry. The correlations between WEE1 expression and clinicopathological features were also studied, as well as the prognosis of patients with CRC.

Materials and methods

Patients and specimens. Matched cancerous and adjacent normal tissue specimens were obtained from 43 patients with CRC who underwent surgery at Guangzhou Red Cross Hospital (Guangdong, China) between March 2012 and January 2013. These specimens were collected and frozen in liquid nitrogen immediately following surgery until RT-qPCR analysis. Additionally, 102 cases of paraffin-embedded CRC tissue specimens (without corresponding normal tissues) were also collected from patients who underwent surgery at the same hospital between March 2008 and December 2009. Clinicopathological data including age, gender, tumor size, tumor histology, lymph node status, tumor node metastasis (TNM) stage and metastasis of all patients in these two cohorts were recorded. Prognostic data including tumor free survival time and overall survival time were also collected for the 102 patients. Prior written informed consent was obtained from all patients involved in the study and the study protocol was approved by the Ethics Committee of Guangzhou Red Cross Hospital. Diagnoses of CRC were all confirmed by histopathological examination.

RNA preparation and RT. Total RNA was extracted from the 43 CRC tissues and the corresponding normal tissues using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol and as described previously (27). Total RNA was reverse transcribed using the PrimeScript™ RT reagent kit (Takara Bio, Inc., Kusatsu, Shiga, Japan) in a final volume of 20 μl containing 4.0 μl of the total RNA sample, 1.0 μl PrimeScript™ RT enzyme mix I, 1.0 μl of Oligo dT primer (50 μM), 2.0 μl of random 6-mers (100 μM), 2.0 μl of 5xPrimeScript™ buffer and 10.0 μl of RNase free distilled H_2O. The RT reaction was performed by a C1000 Thermal cycler (Bio-Rad Laboratories, Inc. Hercules, CA, USA) using the following conditions: 37˚C for 15 min, then 85˚C for 5 sec.

TaqMan-based qPCR for WEE1 mRNA. TaqMan-based qPCR was performed on the extracted RNA using a 7300 Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The PCR assay was carried out in a 25 μl reaction system consisting of 2.0 μl of cDNA sample, 0.5 μl of each primer (forward and reverse, 10 pmol/μl), 0.5 μl of Taq DNA polymerase (Takara Bio, Inc.), 0.5 μl of deoxynucleotide triphosphate mixture (Takara Bio, Inc.), 0.5 μl of probe (5 pmol/μl), 2.5 μl of 10x PCR buffer (Takara Bio, Inc.) and 18.0 μl of distilled H_2O. The PCR amplification consisted of 40 cycles of 93˚C for 15 sec, 55˚C for 25 sec and 72˚C for 25 sec subsequent to an initial denaturation step (93˚C for 2 min). The primers and probes for PCR assay were designed by Primer Express 2.0 (Applied Biosystems; Thermo Fisher Scientific, Inc.) and synthesized by Invitrogen (Thermo Fisher Scientific, Inc.). The TaqMan probe for WEE1 was 5'-FAM-CTGCTG GTGCTGAACCTCTTCC-BHQ1-3'. Primers for WEE1 were forward, 5'-GCTTGCCCTCACAGTGGTATG-3' and reverse, 5'-CCGAGGTAATCTACCCTGTCT-GA-3'. To correct the differences in quality and quantity of complementary DNA (cDNA) samples, β-actin gene was measured in the same samples as an internal control. The TaqMan probe for β-actin was 5'-FAM-CCTCACCCTGAAGTACCCCAT-CGA GC-BHQ1-3'. Primers for β-actin were forward, 5'-GCATGG GTCAGAAGGATTC- CT-3' and reverse, 5'-TCGTCCCAG TTGGTGACGAT-3'. The negative control contained water instead of cDNA. All samples, including the negative control, were analyzed in triplicate.

Quantification for PCR products and score of WEE1 mRNA upregulation. One sample of normal colon mucosa was used as the calibrator to prepare the standard curves for each gene. The target gene of the calibrator was amplified using a qPCR assay with the same conditions as the test samples. The qPCR product was verified by 2% low melting point agarose gel electrophoresis and subsequently extracted and purified using a QIAquick Gel Extraction kit (Qiagen GmbH, Hilden, Germany). The purified product was measured for optical density (OD) 260 and OD 280 and the purity value was satisfactory when the OD 260/OD 280 value was >1.8. The concentration (copy/μl) of the purified product was calculated according to the OD 260 value and the length of the product.

The purified product with a dilution of 1:10 was used as the highest concentration point for the construction of the standard curve. The rest of the standard curve points were prepared by 4 subsequent serial 10x dilutions. All 5 standards were measured with the tissue samples under the same conditions, and the generated standard curve was used to quantify the products of PCR. The relative expression levels of WEE1 mRNA in CRC tissue samples were normalized to the internal control β-actin and calculated as the quantity of WEE1 mRNA (copy/μl)/the quantity of β-actin mRNA (copy/μl). Score of WEE1 mRNA upregulation=the relative expression of WEE1 mRNA in cancer tissue/the relative expression of WEE1 mRNA in the corresponding normal tissue. Score of WEE1 mRNA upregulation was defined as positive when it was >1.5.

Immunohistochemistry analysis. WEE1 protein expression was detected by immunohistochemistry analysis in 102 cases of CRC tissues, in which 43 specimens of CRC were not included. The protocol of immunohistochemistry analysis is described briefly as follows. Tissue sections of 2 μm thickness were cut and baked at 60˚C for 1 h, deparaffinized in xylene and rehydrated through graded ethanol washes (100% ethanol for 3 min, twice; 95% ethanol for 3 min, twice; distilled water for 3 min). Subsequently, sections were subjected to microwave heat-induced antigen retrieval in citrate buffer (0.01 M, pH 6.0; Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China) at high power for 3 min and cooled to

room temperature by the gradual addition of water for at least 20 min.

Subsequent to rinsing with distilled water, 3% hydrogen peroxide was applied to block the endogenous peroxidases at room temperature for 10 min. Sections were then rinsed with PBS 5 times (2 min each time). Samples were incubated at 4˚C overnight with the polyclonal rabbit anti-human WEE1 antibody (dilution, 1:75; catalog no., PAB3322; Abnova, Taipei City, Taiwan). Subsequent to rinsing 5 times with PBS (2 min each time), the sections were incubated with anti-rabbit horseradish peroxide immunoglobulin G (dilution, 1:300; catalog no., SPN-9001; Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd) for 30 min at 37˚C. Slides were then visualized by applying 3,3-diaminobenzidine-tetrahydrochloride for 4 min and then were counterstained with hematoxylin.

One case of hepatocellular carcinoma, with significantly elevated WEE1 protein expression (16), was used as the positive control. The negative control slide was probed with normal goat serum (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd.) under the same experimental conditions. WEE1 staining was examined by counting 200 cells in the region of interest, which focused on tumor cells. No significant necrosis was identified by two independent pathologists who were blind to the clinical characteristics of the samples. The intensity of WEE1 staining was classified using a 4-point scale according to previous literature (18): 0, no positive cell; 1+, <10% positive cells; 2+, 10-50% positive cells; 3+, >50% positive cells. The expression of WEE1 protein was defined as negative if the score was 0 and was classified as positive if the scores were 1+, 2+ or 3+. Based on immunohistochemistry results, patients with CRC were divided into the low WEE1 staining score group (0 or 1+) and the high WEE1 staining score group (2+ or 3+) to compare the prognosis between these two groups.

Statistical analysis. Student's t-test was applied to compare the WEE1 mRNA expression levels in 43 cases of CRC tissues with the corresponding normal tissues. The χ^2 test (or Fisher's exact test, for categorical data) and Student's t-test (for continuous data) were used to analyze the correlations between the scores of WEE1 mRNA upregulation in 43 cases of CRC tissue samples and the clinicopathological characteristics of CRC. The χ^2 test or Fisher's exact test was used to analyze the associations between WEE1 immunostaining scores in 102 cases of CRC samples and the clinicopathological characteristics of CRC. Survival curves were constructed using the Kaplan-Meier method and the differences in tumor-free survival and overall survival were evaluated by a Log-rank test. Univariable Cox regression analysis was employed to explore the prognostic implication of clinicopathological features, including gender, age, maximal tumor size, tumor differentiation, depth of tumor invasion, lymph node metastasis, distant metastasis, TNM stage and WEE1 immunostaining score. Those clinicopathological features associated with prognosis of CRC were subsequently put into a multivariable Cox regression model to identify factors that were independently associated with overall survival rate of CRC. In this model, a step-wise selection was used for variable selection with entry and removal limits of $P \leq 0.05$ and $P > 0.10$, respectively. All statistical analyses were two-sided and performed by SPSS 13.0 software package (SPSS, Inc., Chicago, IL, USA). The continuous data were expressed as the mean ± standard deviation. $P<0.05$ was considered to indicate a statistically significant difference.

Results

The upregulation of WEE1 mRNA in CRC tissues. WEE1 mRNA was detectable in all 43 cases of CRC tissue specimens and its relative expression level in CRC tissues was significantly increased compared with the corresponding adjacent normal tissues (0.404±0.129 vs. 0.289±0.086, $P<0.0001$; Fig. 1). In addition, the score of WEE1 mRNA upregulation in 43 cases of CRC tissue specimens was 1.519±0.773 and positive in 41.9% (18/43) of specimens.

Association between the scores of WEE1 mRNA upregulation and clinicopathological variables of CRC. To explore the clinical association of WEE1 mRNA upregulation in CRC, the clinicopathological data was associated with the scores of WEE1 mRNA upregulation. The present results demonstrated that the scores of WEE1 mRNA upregulation were significantly associated to hepatic metastasis ($P=0.035$), distant metastasis ($P=0.039$) and high TNM stage ($P=0.039$) of patients with CRC (Table I). However, no significant association was identified between the scores of WEE1 mRNA upregulation and the other clinicopathological features, including gender, age, maximal tumor size, tumor differentiation and lymph node metastasis.

Associations between WEE1 immunostaining scores and clinicopathological variables of CRC. As previously described in melanoma and vulvar squamous cell carcinoma (17,18), immunohistochemical staining of WEE1 was predominantly detected in the cellular nucleus despite evidence of positive staining being identified in the cytoplasm. Positive WEE1 expression (Fig. 2A-C) was evidenced in 52.9% (54/102) of patients with CRC. In patients with positive WEE1 staining: 10 patients scored 3+; 19 patients scored 2+; and 25 patients scored 1+. However, WEE1 was undetectable in the remaining 48 patients with CRC and scored 0 (Fig. 2D). When associated with clinicopathological data, WEE1 staining scores were significantly correlated to distant metastasis ($P=0.002$) and a high TNM stage ($P=0.002$) of patients with CRC (Table II). However, no significant association was identified between WEE1 staining scores and the other clinicopathological features, including gender, age, maximal tumor size, tumor differentiation, depth of tumor invasion, and lymph node metastasis, of patients with CRC.

Correlations between WEE1 protein expression and prognosis of patients with CRC. A total of 102 cases of patients with CRC were divided into two groups based on WEE1 expression levels, the high WEE1 staining score group (n=29) and the low WEE1 staining score group (n=73). Patients with CRC within the high WEE1 staining score group had either poorer overall survival (mean overall survival time, 50.552±5.573 vs. 66.280±2.483 months, $P=0.018$; Fig. 3A) or poorer tumor-free survival compared with those within the low WEE1 staining score group (mean tumor-free survival time, 42.999±3.164 vs. 51.266±2.639 months, $P=0.039$; Fig. 3B).

Table I. Association between the scores of WEE1 mRNA upregulation and clinicopathological variables of colorectal cancer.

Clinicopathological variable	n	Upregulation of WEE1 mRNA		P-value
		Positive	Negative	
Gender				0.234[a]
Male	26	9	17	
Female	17	9	8	
Age, years	43	64.8±14.6[b]	71.1±12.2[b]	0.129[c]
Maximal tumor size, mm	43	52.71±23.8[b]	56.9±27.1[b]	0.610[c]
Tumor differentiation				0.234[a]
Well-Moderate	26	9	17	
Poor	17	9	8	
Lymph node metastasis				0.455[a]
Presence	21	10	11	
Absence	22	8	14	
Hepatic metastasis				0.035[a]
Presence	8	6	2	
Absence	35	12	23	
Distant metastasis				0.039[a]
Presence	10	7	3	
Absence	33	11	22	
Tumor node metastasis stage				0.039[a]
I, II, III	33	11	22	
IV	10	7	3	

[a]χ^2 test or Fisher's exact test. [b]Mean ± standard deviation. [c]Student's t-test. Tumor differentiation is according to Japanese Classification of Colorectal Carcinoma (7th edition, 2006). Tumor node metastasis classification is according to International Union against Cancer (7th edition, 2012). P<0.05 was considered to indicate a statistically significant difference. Well, well-differentiated adenocarcinoma; Moderate, moderately-differentiated adenocarcinoma; Poor, poorly-differentiated or mucinous adenocarcinoma.

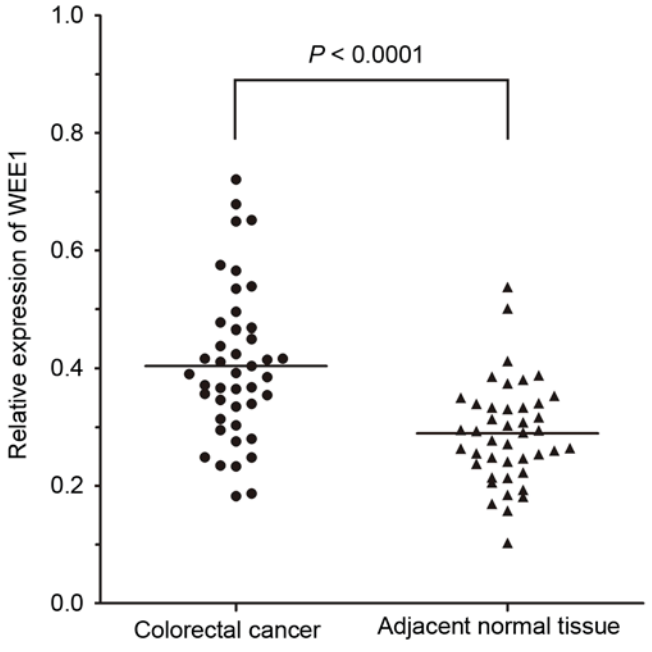

Figure 1. Upregulation of WEE1 in CRC tissues determined by reverse transcription-quantitative polymerase chain reaction. WEE1 expression in CRC tissues was significantly increased compared with that in adjacent normal tissues. Bars in the figure indicate the mean of the relative expression levels of WEE1, P<0.0001. CRC, colorectal cancer.

Independent prognostic implication of high WEE1 staining score for CRC. By using univariable Cox regression analysis, distant metastasis [hazard ratio (HR), 2.823; P=0.015], high TNM stage (HR, 4.382; P=0.005), and high WEE1 staining score (HR, 2.392; P=0.023) were associated with the overall survival rate of patients with CRC (Table III). A total of 5 variables including gender, age, maximal tumor size, tumor differentiation, and lymph node metastasis did not enter the multivariable Cox regression model. It is noteworthy that in this multivariable model, only high WEE1 expression (HR, 3.339; P=0.039) and high TNM stage (HR, 5.126; P=0.024) were identified to be independent prognostic factors for patients with CRC (Table III).

Discussion

Although the upregulation of WEE1 has been observed in several human malignancies, including in HCC and melanoma, and in numerous types of tumor cell lines including glioblastoma and breast cancer (16-19), the data regarding the expression pattern of WEE1 in human CRC remains limited. Using a cDNA array and semi-quantitative RT-PCR, Backert *et al* (26) identified that WEE1 expression was decreased in human colon cancer cell lines. This

Table II. Association between the WEE1 immunostaining scores and clinicopathological variables of colorectal cancer.

Clinicopathological variable	n	WEE1 immunostaining scores				P-value
		0	1+	2+	3+	
Gender						0.607
Male	62	31	16	9	6	
Female	40	17	9	10	4	
Age, years						0.590
≤65	70	30	18	15	7	
>65	32	18	7	4	3	
Maximal tumor size[a]						0.351
Mean ≤43.6 mm	57	23	17	12	5	
Mean >43.6 mm	45	25	8	7	5	
Tumor differentiation						0.134
Well-Moderate	89	43	24	14	8	
Poor	13	5	1	5	2	
Depth of tumor invasion						0.816
≤Mt	20	9	6	4	1	
>Mt	82	39	19	15	9	
Lymph node metastasis						0.071
Presence	30	11	5	9	5	
Absence	72	37	20	10	5	
Distant metastasis						0.002
Presence	13	1	3	5	4	
Absence	89	47	22	14	6	
TNM stage						0.002
Stage I, II, III	89	47	22	14	6	
Stage IV	13	1	3	5	4	

[a]Tumor sizes were divided by mean of all patients. Tumor differentiation and the depth of tumor invasion are according to Japanese Classification of Colorectal Carcinoma (7th edition, 2006). TNM classification is according to International Union against Cancer (7th edition, 2012). P<0.05 was considered to indicate a statistically significant difference. Well, well-differentiated adenocarcinoma; moderate, moderately-differentiated adenocarcinoma; Poor, poorly-differentiated, mucinous or signet ring cell adenocarcinoma; Mt, muscular tunic; TNM, tumor node metastasis.

downregulation was verified in 7 cases of CRC tissues despite the evidence that WEE1 was only detectable in 6 cases of normal tissues and 3 cases of CRC tissue. Considering the difference between cancer tissues and cell lines, the small number of tissue samples tested and the low sensitivity and accuracy of conventional RT-PCR, it appears that the data provided by Backert *et al* (26) were not enough to ascertain the expression pattern of WEE1 in CRC tissues. The present study therefore employed a quantitative TaqMan-based RT-qPCR analysis to detect the expression levels of WEE1 in 43 cases of CRC tissues and matched adjacent normal tissues. The present results demonstrated that WEE1 mRNA was detectable in all CRC tissue and adjacent normal tissue specimens tested, showing the higher sensitivity of RT-qPCR compared with conventional RT-PCR. Notably, WEE1 mRNA expression was significantly increased in CRC tissues compared with the corresponding adjacent normal tissues and the upregulation of WEE1 mRNA was observed in 41.9% of patients with CRC (18/43 cases). The upregulation of WEE1 mRNA in CRC is not only in agreement with the expression pattern of WEE1 in other human malignancy tissues, including HCC (16-19), but also suggests WEE1 upregulation as a common event during the carcinogenesis of human malignancies, including CRC.

Although the exact role of WEE1 in human malignancy still needs further studies in order to be understood, Magnussen *et al* (18) previously identified that high WEE1 expression is associated with lymph node metastasis and poor differentiation of vulvar squamous cell carcinoma. The present study therefore correlated WEE1 mRNA upregulation with clinicopathological characteristics of CRC and revealed that the upregulation of WEE1 mRNA was significantly correlated with the distant metastasis of CRC. In addition, there was also an association between WEE1 mRNA upregulation and hepatic metastasis of CRC. Previously, WEE1 expression was increased in metastatic melanomas compared with primary melanomas (17). Huisman *et al* (28) also reported that expression rhythm of WEE1, a circadian clock-controlled gene (29), was completely disrupted in colorectal liver metastases. Based on these data, the present study presumed that WEE1

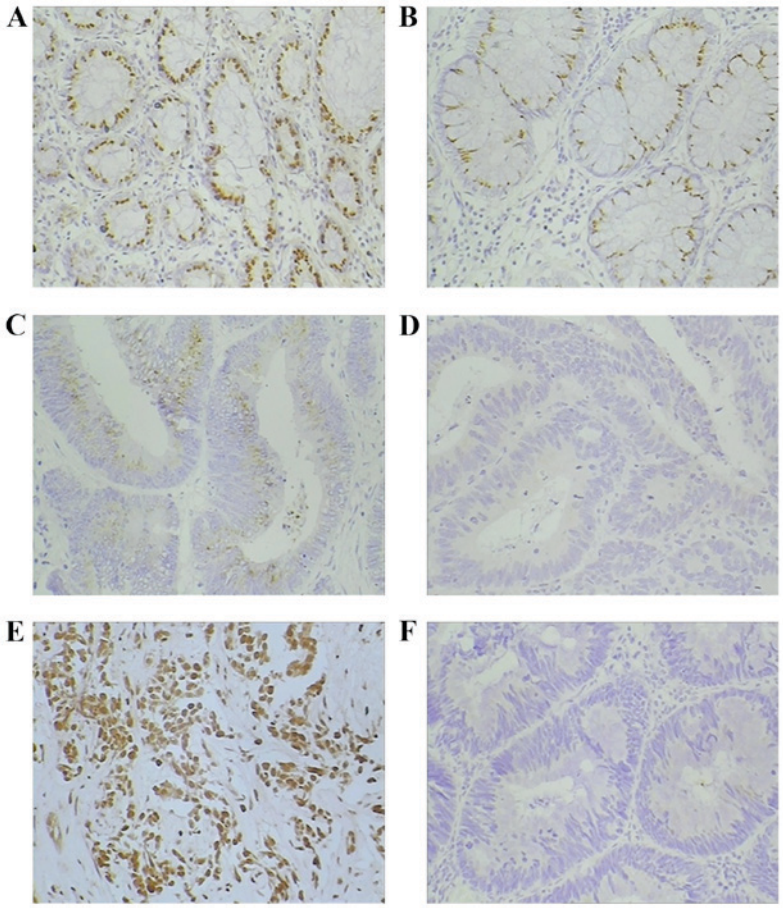

Figure 2. Immunostaining of WEE1 protein in colorectal cancer tissues. Representative WEE1 staining in nuclei are presented and scored as (A) 3+, (B) 2+, (C) 1+, and (D) 0. (E) One case of hepatocellular carcinoma was used as the positive control. (F) The negative control slide was probed by normal rabbit serum substituting for anti-WEE1 antibody. Original magnification for all images, x400.

upregulation may be involved in the metastasis potential of colon cancer, however this should be verified by future studies. Since distant metastasis is an important parameter in the TNM stage system of CRC (30), the present study identified that WEE1 was more strongly upregulated in patients with CRC with a high TNM stage (stage VI) than those with a low TNM stage (stage I-III). Together, these results indicate that upregulation of WEE1 mRNA is closely associated with a high degree of malignancy in CRC.

Subsequently, the present study determined the expression pattern of WEE1 protein in 102 cases of CRC by immunohistochemistry analysis. The present results demonstrated that WEE1 staining was predominantly observed in the cellular nucleus although a limited amount of staining was also identified in cytoplasm, indicating WEE1 as a mainly nucleus-located protein similar to the location of WEE1 in vulvar squamous cell carcinoma (18). The present study also revealed that WEE1 was positive in 52.9% of patients with CRC, which is lower than the positive rate of WEE1 in melanoma and vulvar squamous cell carcinoma tissues (17,18) and suggested the different expression level of WEE1 in different human tissues. When correlated to clinicopathological data WEE1 protein staining scores were significantly associated with the distant metastasis of CRC and high TNM stage. Additionally, there was a trend in association with WEE1 protein staining scores and lymph node metastasis of CRC (P=0.071). The present results therefore increase the evidence for the involvement of WEE1 in the malignancy progression of CRC.

It has previously been suggested that high WEE1 expression was associated with poor disease-free survival of malignant melanoma (17). Therefore, the present study divided the 102 cases of CRC into a low WEE1 expression group and a high WEE1 expression group based on immunohistochemistry results to explore the prognostic implication of WEE1 expression. The present results revealed that patients with CRC within the high WEE1 expression group had either a poorer disease-free survival or overall survival compared with those within the low WEE1 expression group. These results are consistent with results obtained from studies on malignant melanoma, increasing evidence for the potential prognostic value of high WEE1 expression to CRC. Slipicevic *et al* (19) identified WEE1 as a novel independent prognostic marker of poor survival for ovarian carcinoma patients following chemotherapy by multivariate Cox analysis. Therefore, the present study also established a Cox regression model, and this model indicated high WEE1 expression to be an independent risk factor for the prognosis of patients with CRC.

In conclusion, the present study revealed that WEE1 is upregulated in human CRC tissues and the increased WEE1 expression is correlated with a high degree of malignancy and poor survival of patients with CRC, which suggests WEE1 as a novel prognostic marker for CRC. However, further studies

Table III. Univariable and multivariable Cox analysis for the prognostic factors of colorectal cancer.

		Univariable analysis		Multivariable analysis	
Variable	n	HR (95% CI)	P-value	HR (95% CI)	P-value
Gender					
Male	62	1	0.549	-	-
Female	40	0.797 (0.379-1.675)			
Age (year, continuous data)	102	1.117 (0.492-2.537)	0.791	-	-
Maximal tumor size (mm, continuous data)	102	0.998 (0.979-1.018)	0.857	-	-
Tumor differentiation					
Well-mod	89	1	0.350	-	-
Poor	13	1.987 (0.471-8.372)			
Depth of tumor invasion					
≤Mt	20	1	0.596	-	-
>Mt	82	1.006 (0.512-1.897)			
LN metastasis					
Absence	72	1	0.217	-	-
Presence	30	1.019 (0.989-1.051)			
Distant metastasis					
Absence	89	1	0.015[a]	1	0.095
Presence	13	2.823 (1.055-7.632)		3.327 (0.855-9.763)	
TNM stage					
Stage I, II, III	89	1	0.005[a]	1	0.024
Stage IV	13	4.382 (1.595-7.258)		5.126 (1.176-8.511)	
WEE1 staining score					
Low staining score	73	1	0.023[a]	1	0.039
High staining score	29	2.392 (1.130-5.062)		3.339 (1.030-9.552)	

[a]Tumor differentiation and the depth of tumor invasion are according to Japanese Classification of Colorectal Carcinoma (7th edition, 2006). Tumor node metastasis classification is according to International Union against Cancer (7th edition, 2012). P<0.05 was considered to indicate a statistically significant difference. HR, hazard ratio; CI, confidence interval; well, well-differentiated adenocarcinoma; mod, moderately-differentiated adenocarcinoma; Poor, poorly-differentiated, mucinous or signet ring cell adenocarcinoma; LN, lymph node; TNM, tumor node metastasis.

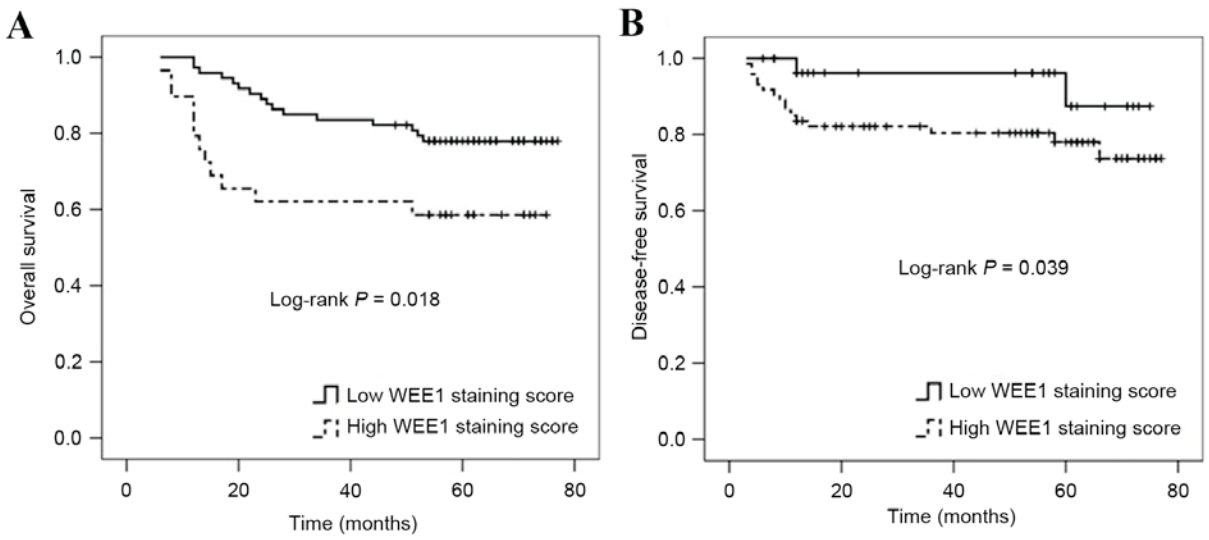

Figure 3. Estimated survival according to the staining scores of WEE1 in 102 cases of patients with CRC (by Kaplan-Meier method). Log-rank test shows that patients with CRC within the high WEE1 staining score group exhibit either (A) poorer overall survival or (B) poorer disease-free survival compared with those within low WEE1 staining score group. CRC, colorectal cancer.

are still required to elucidate the mechanisms underlying the upregulation of WEE1 in patients with CRC.

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