Overexpression of the Wilms' tumor gene *WT1* in colorectal adenocarcinoma

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Expression of the Wilms' tumor gene *WT1* was examined in 59 cases of colorectal adenocarcinoma to examine the involvement of *WT1* in tumorigenesis. Quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) showed that *WT1* mRNA was expressed in the range from 7.2×10^{-5} to 4.9×10^{-1} levels (*WT1* expression level in K562 leukemic cells was defined as 1.0) in all (100%) of the 28 cases of colorectal adenocarcinoma examined, and that the *WT1* mRNA expression levels were higher in 20 (71%) of the 28 cases compared to those of normal-appearing mucosal tissues examined. Immunohistochemical analysis using an anti-WT1 antibody was performed on 46 cases of colorectal adenocarcinoma (15 of the 28 cases with *WT1* mRNA expression and 31 newly collected cases), and the expression of WT1 protein was detected in 41 (89%) of the 46 cases. The direct sequencing analysis of the *WT1* genomic DNA showed no mutations in any of the 10 exons of the *WT1* gene in any of 5 different colorectal adenocarcinomas. These results may indicate an important role of the wild-type *WT1* gene in tumorigenesis of colorectal adenocarcinoma. (Cancer Sci 2003; 94: 712–717)

The Wilms' tumor gene (*WT1*) was originally isolated as a tumor-suppressor gene that was inactivated in a subset of Wilms' tumors and mutated in the germline of children with a genetic predisposition to this kidney neoplasm of the childhood.[1–3] The *WT1* gene encodes a zinc finger transcription factor that regulates transcription of growth factor (*PDGF-A chain*, *CSF-1*, and *IGF-II*)[4–6] and growth factor receptor (*IGF-IR*)[7] genes and other genes (*RAR-α*, *c-myc*, and *bcl-2*).[8, 9]

We have demonstrated that the wild-type *WT1* gene is expressed in cancer cells derived from various kinds of cancers, including colon cancer,[10, 11] and overexpressed in primary leukemia,[12] breast,[13, 14] lung cancer,[15] bone and soft-tissue sarcoma,[16] and head and neck squamous cell carcinoma (HNSCC).[17] Furthermore, growth of *WT1*-expressing cancer cells was inhibited by treatment with *WT1* antisense oligomers.[10, 18, 19] Therefore, we proposed that the wild-type *WT1* gene plays an oncogenic role rather than having a tumor-suppressor function in tumorigenesis of various types of cancers.[20]

Colorectal adenocarcinoma is a common disease in both men and women and an important public health problem worldwide. Many genes, including oncogenes such as *myc*[21, 22] and *ras*,[23, 24] and tumor suppressor genes, such as *p53*[25, 26] and *APC*,[27] have been reported to be involved in the pathogenesis of colorectal adenocarcinoma. However, the precise mechanism of tumorigenesis of colorectal adenocarcinoma remains unclear.

In the present study, we analyzed expression of the *WT1* gene in human primary colorectal adenocarcinoma to examine the involvement of WT1 in tumorigenesis of colorectal adenocarcinoma, and found that the *WT1* gene was expressed at the mRNA level in all (100%) of 28 cases and overexpressed at the protein level in 41 (89%) of 46 cases of colorectal adenocarcinoma examined.

Materials and Methods

Tissue samples. WT1 expression was analyzed in 59 cases of colorectal adenocarcinoma. In 28 cases, expression levels of the *WT1* gene were determined by quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR). In 46 cases (15 of the 28 cases above and 31 newly collected cases), WT1 protein expression in colorectal adenocarcinoma tissue was analyzed by immunohistochemistry. For real-time RT-PCR analysis, paired colorectal adenocarcinoma tissues and normal-appearing colorectal mucosal tissues more than 5 cm apart from the margin of the tumors were obtained. All samples were obtained with informed consent at Osaka University Hospital and Nissay Hospital. Clinicopathological features of the 59 cases are shown in Table 1.

RNA purification and RT-PCR. Total RNA was isolated from the sample tissues using Trizol (Invitrogen, Leek, the Netherlands) according to the manufacturer's instructions, dissolved in diethylpyrocarbonate (DEPC)-treated water, and quantified in terms of the absorbance at 260 nm with a spectrophotometer. RNA was converted into cDNA as described previously[15] and stored at −20°C until use.

To determine relative *WT1* expression levels, cDNA (3.0 μl for *WT1* and 2.0 μl for β-actin) was added to the PCR buffer (100 m*M* Tris-HCl, pH 8.3; 500 m*M* KCl; and 3 m*M* $MgCl_2$) containing 200 μ*M* of each dNTP, 1.25 U of Ampli*Taq* Gold (PE Applied Biosystems, Foster City, CA), 0.5 μ*M* forward and reverse primers, and 200 n*M* *Taq*Man probe in a total volume of 50 μl. The sequences of primers and probes used are as follows. *WT1*: forward primer (F1), 5′GATAACCACACAACGCCCATC3′; reverse primer (R1), 5′CACACGTCGCACATCCTGAAT3′; probe, 5′FAM-ACACCGTGCGT GTGTATTCTGTATTGG-TAMRA3′. β-Actin: forward primer, 5′CCCAGCACAATGA AGATCAAG ATCAT3′; reverse primer, 5′ATCTGCTGGAAGGTGGACAGCGA3′; probe, 5′FAM-TGAGCGCAAGTACTC CGTGTGGATCGGCG-TAMRA3′. After activation of Ampli*Taq* Gold polymerase at 95°C for 10 min, PCR was performed for 40 cycles (95°C for 30 s/63°C for 60 s). Sequences of *WT1* reverse and β-actin forward primers spanned two consecutive exons from exons 6 to 7 and from exons 4 to 5 of the respective gene in order to avoid amplification of the corresponding genome sequences. Standard curves for the quantitation of *WT1* and β-actin were constructed from the re-

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Table 1. Clinical features of patients with colorectal adenocarcinoma and *WT1* expression in cancer tissues

Pt. ID	Age (year)	Gender	Site	Differentiation	TNM classification	Stage	*WT1* mRNA level	WT1 protein
1	73	M	rectum	mod	T2N0M0	I	5.6×10^{-2}	++
2	54	F	rectum	mod	T2N0M0	II	2.6×10^{-2}	n.d.
3	80	M	rectum	well	T4N2M0	IIIb	2.1×10^{-2}	++
4	55	F	rectum	mod	T2N0M0	I	1.4×10^{-2}	n.d.
5	68	M	rectum	mod	T2N2M0	IIIb	9.5×10^{-3}	n.d.
6	72	M	rectum	well	T2N0M0	I	5.6×10^{-3}	++
7	52	F	rectum	well	T3N1M0	IIIa	5.6×10^{-3}	+
8	88	F	rectum	well	T3N0M0	II	3.5×10^{-3}	++
9	65	M	rectum	mod	T1N0M0	I	3.4×10^{-3}	n.d.
10	44	F	rectum	well	T2N0M0	I	2.3×10^{-3}	++
11	35	M	rectum	mod	T2N3M1 (HEP)	IV	2.2×10^{-3}	n.d.
12	60	M	rectum	well	T3N0M0	II	1.5×10^{-3}	n.d.
13	78	F	rectum	mod	T2N1M0	IIIa	7.3×10^{-4}	n.d.
14	62	M	rectum	mod	T2N1M0	IIIa	5.6×10^{-4}	n.d.
15	53	M	rectum	mod	T2N0M0	II	5.1×10^{-4}	n.d.
16	51	M	rectum	mucinous	T3N1M0	IIIa	2.1×10^{-4}	++
17	66	M	rectum	well	T3N0M0	II	2.0×10^{-4}	++
18	57	F	rectum	mucinous	T3N0M0	II	9.5×10^{-5}	++
19	81	M	rectum	well	T2N0M0	II	n.d.	++
20	66	F	rectum	mod	T1N1M0	IIIa	n.d.	++
21	64	M	rectum	mod	T2N1M1 (HEP)	IV	n.d.	++
22	44	F	rectum	mod	T2N0M0	II	n.d.	++
23	52	F	rectum	mod	T2N1M0	IIIa	n.d.	++
24	73	F	rectum	mod	T2N1M1 (HEP)	IV	n.d.	++
25	66	M	rectum	mod	T2N2M1 (PUL)	IV	n.d.	++
26	55	F	rectum	mod	T2N1M0	IIIa	n.d.	++
27	63	M	rectum	mucinous	T2N0M0	II	n.d.	++
28	62	M	rectum	mod	T2N1M0	IIIa	n.d.	++
29	71	F	rectum	mod	T2N1M0	IIIa	n.d.	++
30	52	F	rectum	mod	T2N0M0	II	n.d.	++
31	53	F	rectum	mod	T3N0M0	IIIa	n.d.	++
32	53	M	rectum	mod	T2N0M0	II	n.d.	++
33	59	F	rectum	mucinous	T2N0M0	II	n.d.	++
34	69	F	rectum	mod	T3N0M0	II	n.d.	++
35	68	F	rectum	mod	T2N2M0	IIIb	n.d.	++
36	87	M	rectum	mod	T2N1M0	IIIa	n.d.	–
37	56	M	rectum	mod	T2N1M0	IIIa	n.d.	–
38	53	M	sigmoid colon	well	T1N0M0	I	6.9×10^{-4}	–
39	62	F	sigmoid colon	well	T2N1M0	IIIa	7.2×10^{-5}	++
40	49	M	sigmoid colon	mod	T2N0M1 (HEP)	IV	n.d.	++
41	70	M	sigmoid colon	mod	T2N0M0	II	n.d.	++
42	61	M	sigmoid colon	mod	T2N0M0	II	n.d.	++
43	59	M	sigmoid colon	mod	T2N1M1 (HEP)	IV	n.d.	++
44	70	M	sigmoid colon	mod	T2N0M0	II	n.d.	++
45	60	M	sigmoid colon	mod	T2N0M0	II	n.d.	+
46	59	M	descending colon	mod	T2N0M1 (HEP)	IV	7.4×10^{-3}	n.d.
47	75	F	descending colon	well	T2N0M0	I	4.3×10^{-3}	+
48	63	M	descending colon	mod	T2N1M1 (HEP)	IV	2.1×10^{-3}	n.d.
49	54	F	descending colon	mod	T3N1M1 (HEP, PUL)	IV	1.3×10^{-3}	++
50	73	M	transverse colon	well	T3N2M0	IIIb	1.7×10^{-2}	++
51	49	M	ascending colon	poor	T3N1M0	IIIa	4.9×10^{-1}	++
52	67	F	ascending colon	mod	T2N2M1 (PER)	IV	1.8×10^{-3}	n.d.
53	69	M	ascending colon	mod	T3N0M0	II	1.6×10^{-3}	n.d.
54	60	M	ascending colon	mod	T2N0M0	II	n.d.	++
55	82	F	ascending colon	mod	T2N0M0	II	n.d.	++
56	82	F	ascending colon	mod	T3N1M1 (HEP)	IV	n.d.	++
57	69	F	ascending colon	well	T2N1M1 (HEP)	IV	n.d.	+
58	76	F	ascending colon	mod	T2N1M0	IIIa	n.d.	–
59	71	M	ascending colon	mod	T2N0M0	II	n.d.	–

WT1 mRNA expression levels were determined by real-time RT-PCR. *WT1* mRNA expression level in leukemic cell line K562 cells was defined as 1.0. WT1 protein expression was determined by immunohistochemistry. ++, positive; +, weakly positive; –, negative; n.d., not determined.

sults of simultaneous amplification of serial dilutions of the cDNA from *WT1*-expressing K562 leukemic cells, whose *WT1* expression level was defined as 1.0, as described previously.[15] Real-time PCR and subsequent calculations were performed on an ABI Prism 7700 Sequence Detector System (PE Applied Biosystems). To normalize the differences in RNA degradation and in RNA loading for RT-PCR in individual samples, the values of levels of *WT1* gene expression divided by those of *β-actin* gene expression were defined as relative *WT1* expression levels in the samples. All experiments were performed in duplicate.

Immunohistochemistry. Formalin-fixed tissue sections of 4-μm thickness were cut from each paraffin block. After dewaxing with xylene and rehydration through a graded series of ethanol, the sections were microwaved for 15 min in 10 m*M* citrate buffer (pH 6.0) for antigen retrieval. After blocking of endogenous biotin using the Biotin Blocking System (DAKO, Glostrup, Denmark), these sections were incubated in phosphate-buffered saline containing goat serum albumin, reacted with anti-WT1 rabbit polyclonal antibody C-19 (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:100 at 4°C overnight and then reacted with biotinylated goat anti-rabbit IgG antibody (Vector Labs., Burlingame, CA) diluted 1:100 at 37°C for 30 min. After treatment with 3% H_2O_2 solution to reduce endogenous peroxidase activity, immunoreactive WT1 protein was visualized using a Vectastain ABC kit (Vector Labs.) according to the manufacturer's instructions. The sections were then counterstained with methyl green.

Sequencing analysis. Genomic DNA was isolated from the frozen colorectal adenocarcinoma tissues with standard techniques, dissolved in distilled water, and quantified in terms of the absorbance at 260 nm with a spectrophotometer. For amplification of exons 2–10 of the *WT1* gene, 0.2 μg of genomic DNA was added to the PCR buffer (100 m*M* Tris-HCl, pH 8.3; 500 m*M* KCl; and 3 m*M* $MgCl_2$) containing 250 μ*M* of each dNTP, 1.25 U of Ex*Taq* polymerase (TAKARA, Shiga), and 0.5 μ*M* forward and reverse primers[15, 17, 28] (Table 2) in a total volume of 50 μl. For amplification of exon 1 of the *WT1* gene that has high GC content, 0.2 μg of genomic DNA was added to the PCR buffer (1× Pfx amplification buffer with 2.5 m*M* $MgCl_2$ and 1× PCRx enhancer solution) containing 250 μ*M* of each dNTP, 1.25 U of PLATINUM Pfx DNA polymerase (Invitrogen), and 0.5 μ*M* forward (A-1) and reverse (AA-2) primers (Table 2) in a total volume of 50 μl. PCR amplification was carried out using a thermal cycler TP-3000 (TAKARA) for 35 cycles; each cycle consisting of 60 s at 94°C, 60 s at 53°C for exon 1 or 55°C for exons 2–10, and 90 s at 72°C. PCR products were separated on 2% agarose gel, cut out from the gel, and purified using a Qiaquick gel extraction kit (Qiagen, Valencia, CA). After ethanol precipitation, the PCR-amplified DNA fragments were directly sequenced in both directions by an ABI Prism 377 sequencer (Perkin Elmer Life Science, Boston, MA) using appropriate primers (Table 2) and a Big Dye Terminator Cycle Sequencing Kit (Perkin Elmer Life Science).

Statistical analysis. Statistical analysis to assess correlations between *WT1* expression levels in colorectal cancer and the clinical parameters was performed using the unpaired *t* test or one-way factorial analysis of variance (ANOVA). Fisher's protected least significant difference (PLSD) was used as a post hoc test.

Table 2. Primers for amplification and sequencing of exons of *WT1* genomic DNA

Exons	Primers	
	Names	Sequences
1	A-1	5′-GGAATTCAGCAAATGGGCTCCGACGTG-3′
	A-2	5′-GTAAGCCGAAGCGCCCG-3′
	AA-1	5′-CCGGTGCTGGACTTTGCG-3′
	AA-2	5′-CCTGAATTCCCGGCCTACTTACCC-3′
2	B-1	5′-CCCAAGCTTCCGTCTTGCGAGAGCACC-3′
	B-2	5′-CCCCGAATTCAATTTGCTGTGGGTTAGG-3′
3	C-1	5′-CCCCAAGCTTCTCGTGTCTCCCCCAAC-3′
	C-2	5′-CGAATTCAGCCTCCAAGACCCAGCATGC-3′
4	DD-1	5′-GTGTATAACTGTGCAGAGATCAGTGG-3′
	DD-2	5′-GTCACAGAGAGCTTTGCCCTTTCTTC-3′
5	E-1	5′-CCTGAATTCCACTCCCCACCTCTTC-3′
	E-2	5′-CCTGAATTCGCCATTTGCTTTGCC-3′
6	F-1	5′-CCTGAATTCCTTTTTCCCTTCTTTG-3′
	F-2	5′-CCTGAATTCCTTCCGCTGGGGCC-3′
7	G-1	5′-CCTGAATTCGCTTAAAGCCTCCCTTC-3′
	G-2	5′-CCTGAATTCTTGAACCATGTTTGCCC-3′
8	H-1	5′-CCTGAATTCGAGATCCCCTTTTCCAGT-3′
	H-2	5′-CCTGAATTCACAGCTGCCAGCAATG-3′
9	I-1	5′-CCTGAATTCTCACTGTGCCCACATTG-3′
	I-2	5′-CCTGAATTCAATTTCATTCCACAATAG-3′
10	J-1	5′-CCTGAATTCCTGTCTCTTTGTTGC-3′
	J-2	5′-GTCCCCGAGGGAGACCCC-3′

Results

***WT1* mRNA expression in colorectal adenocarcinoma.** *WT1* gene expression levels in 28 cases of colorectal adenocarcinoma were examined by means of quantitative real-time RT-PCR. As shown in Table 1 and Fig. 1, the *WT1* gene was expressed at the levels ranging from 7.2×10^{-5} to 4.9×10^{-1} in all of the 28 cases of colorectal adenocarcinoma examined. In normal-appearing colorectal mucosal tissues, *WT1* was detected in 9 of 12 cases at low levels ranging from 1.9×10^{-5} to 8.9×10^{-4} and was undetectable in the remaining 3. The *WT1* mRNA expression levels in carcinoma tissues were significantly higher than those in normal-appearing colorectal mucosal tissues ($P<0.0001$).

Expression of WT1 protein in colorectal adenocarcinoma. Expression of WT1 protein was examined by immunohistochemistry in 46 cases of colorectal adenocarcinoma. They included 28 rectal adenocarcinoma and 8 sigmoid, 2 descending, one transverse, and 7 ascending colonic adenocarcinomas. In all sections, normal colorectal mucosal cells could be seen adjacent to

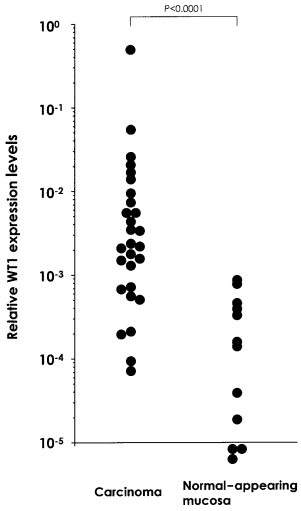

Fig. 1. Overexpression of the *WT1* gene in colorectal adenocarcinoma. Relative *WT1* mRNA expression levels in colorectal adenocarcinoma and normal-appearing colorectal mucosal tissues were examined by a quantitative real-time RT-PCR method. *WT1* expression level in leukemia cell line K562 was defined as 1.0.

carcinoma cells. The intensity of the staining of stained carcinoma cells was scored as one of three categories: negative (−) (negative or weakly positive staining in carcinoma cells similar to that in normal mucosa cells); weakly positive (+) (slightly increased staining of carcinoma cells compared to normal mucosal cells); and positive (++) (markedly increased staining of carcinoma cells compared to normal mucosal cells). Of 28 rectal adenocarcinomas, 25 were scored as positive and one as weakly positive, but the remaining two as negative. Of 8 cases of sigmoid colonic adenocarcinoma, 6 were scored as positive and one as weakly positive, but the remaining one as negative. Of two cases of descending colonic adenocarcinoma, one was scored as positive and the other as weakly positive. One case of transverse colonic adenocarcinoma was scored as positive. Of 7 cases of ascending colonic adenocarcinoma, 4 cases were scored as positive and one as weakly positive, but the remaining 2 cases as negative. Thus, of 46 cases of colorectal adenocarcinoma, 37 (80%) cases were scored as positive and 4 (9%) as weakly positive, but the remaining 5 (11%) as negative (Tables 1 and 3). These results showed that the *WT1* gene was overexpressed at the protein level in the majority of colorectal adenocarcinomas. Representative results are shown in Fig. 2.

Table 3. Expression of WT1 protein in colorectal adenocarcinoma

Site of tumors	No. of cases examined	Expression of WT1 protein		
		++	+	−
rectum	28	25	1	2
sigmoid colon	8	6	1	1
descending colon	2	1	1	0
transverse colon	1	1	0	0
ascending colon	7	4	1	2
Total	46	37 (80%)	4 (9%)	5 (11%)
		41 (89%)		

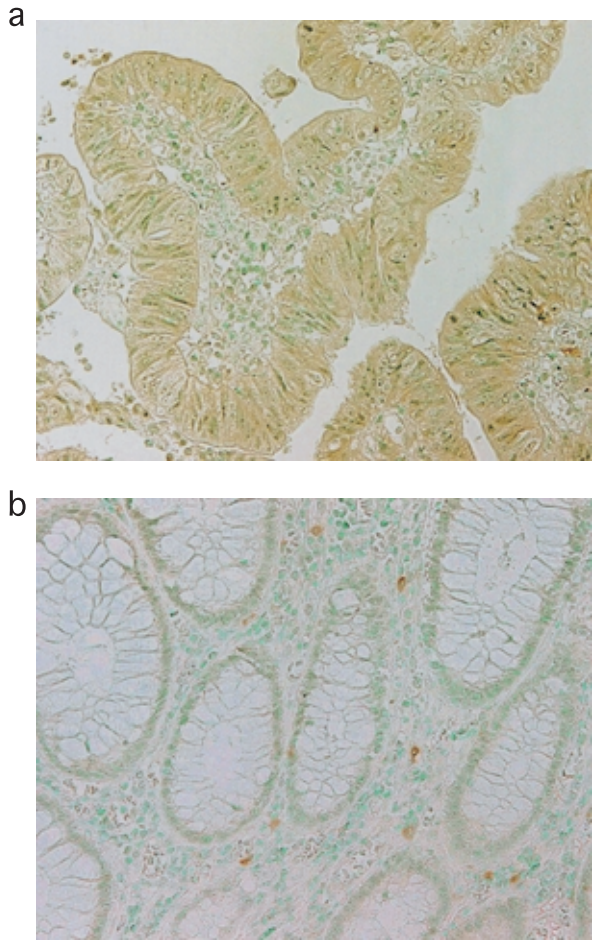

Fig. 2. Expression of WT1 protein in sigmoid colonic adenocarcinoma. Sectioned sigmoid colonic adenocarcinoma tissue of patient 39 was stained with an anti-WT1 antibody. Cytoplasmic staining of WT1 protein was observed with brown in carcinoma cells (a), but not in normal mucosal cells (b).

Correlation between *WT1* expression levels and clinical parameters. Whether or not *WT1* mRNA expression levels correlated with clinicopathological parameters was statistically analyzed. No significant correlations were observed between *WT1* mRNA expression levels and any of age, gender, site of tumors, T stage, N stage, M stage and clinical stage (Table 4 and Fig. 3, A and B).

Absence of mutations in the *WT1* gene in colorectal adenocarcinoma. To determine whether or not the *WT1* gene overexpressed in these colorectal adenocarcinoma had mutations, the *WT1* genomic DNA from 5 cases (patients 1, 3, 4, 6, and 50) was PCR-amplified and examined for mutations by direct sequencing. The sequencing analysis demonstrated the absence of mutations throughout the 10 exons of the *WT1* gene in all of 5 different cases of colorectal adenocarcinoma. Two different single nucleotide polymorphisms (SNP) that did not alter the amino acid sequence of WT1 protein were detected in the *WT1* gene. A polymorphism, Pro42, C→T in exon 1 was detected in 4 (patients 3, 4, 6, and 50) of the 5 cases examined. In the remaining one case (patient 1), Pro42, C→T in exon 1 and Arg300, A→G in exon 7 were detected (data not shown).

Table 4. Correlations between *WT1* mRNA expression levels and clinicopathological characteristics in colon cancer

Clinicopathological characteristics	Total	*P* value
All cases	28	
Sex		
Male	17	0.4470[a]
Female	11	
Age		
<60 years	12	0.8669[a]
≥60 years	16	
Site of tumors		
rectum	18	0.2048[b]
sigmoid colon	2	
descending colon	4	
transverse colon	1	
ascending colon	3	
T stage		
T1	2	0.711[b]
T2	15	
T3	10	
T4	1	
N stage		
N0	15	0.5625[a]
N1–N3	13	
M stage		
M0	23	0.9149[a]
M1	5	
Clinical stage		
I	7	0.5471[b]
II	7	
III	9	
IV	5	

Statistical analysis was performed by unpaired *t* test (a) or ANOVA (b).

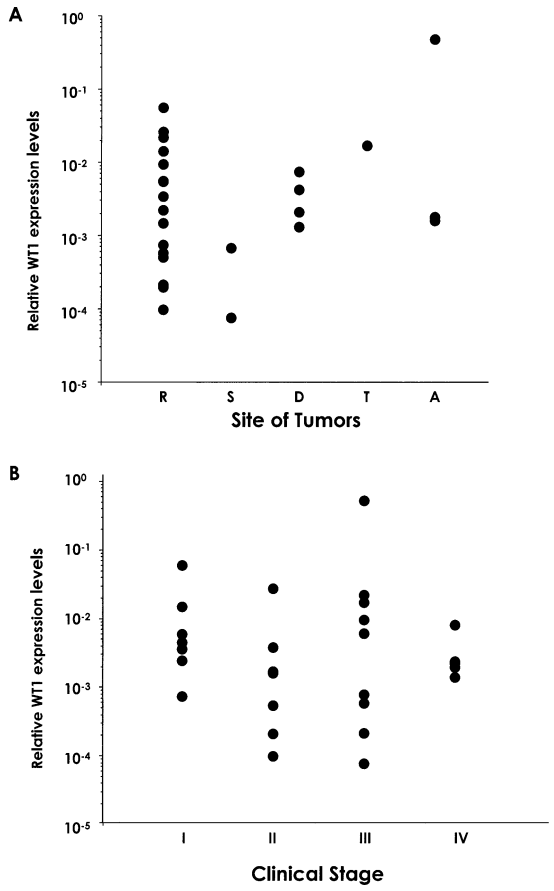

Fig. 3. Correlation between *WT1* mRNA expression levels and clinical parameters of colorectal adenocarcinoma. A, Correlation between *WT1* mRNA expression levels and site of tumors. R, rectum; S, sigmoid colon; D, descending colon; T, transverse colon; A, ascending colon. B, Correlation between *WT1* mRNA expression levels and clinical stages.

Discussion

The *WT1* gene was originally isolated as a tumor suppressor gene responsible for Wilms' tumor, a kidney neoplasm of childhood. However, we hypothesized that the *WT1* gene plays an oncogenic role in tumorigenesis of various types of cancers on the basis of the following findings[20]: (a) the wild-type *WT1* gene was overexpressed in leukemia,[12] breast,[13, 14] lung cancer,[15] and bone and soft-tissue sarcoma,[16] and HNSCC,[17] (b) high expression levels of *WT1* mRNA significantly correlated with poor prognosis in leukemia[12] and breast cancer,[14] and with high tumor-stage in testicular germ-cell tumors[29] and HNSCC,[17] (c) growth of *WT1*-expressing cancer cells was inhibited by treatment with *WT1* antisense oligomers,[10, 18, 19] (d) constitutive expression of *WT1* blocked differentiation, and instead induced proliferation in response to granulocyte colony-stimulating factor (G-CSF) in 32D cl3 myeloid progenitor cells[30] and normal myeloid progenitor cells.[31] The present study demonstrated that the *WT1* gene was overexpressed in the majority of the primary colorectal adenocarcinomas examined and that the *WT1* gene overexpressed in colorectal adenocarcinoma was the non-mutated, wild-type. These results supported our hypothesis, indicating that the wild-type *WT1* gene does play an oncogenic role in the tumorigenesis of human primary colorectal adenocarcinoma.

The *WT1* expression levels were widely distributed in normal-appearing colorectal mucosal tissues examined and more than half of these tissues expressed the gene at levels similar to those in colorectal carcinoma. One possibility was the presence of WT1-expressing pre-malignant cells in normal-appearing colorectal mucosal tissues. Fearon and Vogelstein described a multistep model of the tumorigenesis of colon cancer.[32] According to this model, genetic events such as *ras* mutation and loss of *APC, DCC*, and *p53* accumulate in affected colonic cells and give rise to carcinoma. In the present study, overexpression of the *WT1* gene was observed in most of the colorectal adenocarcinomas examined. This high frequency of *WT1* overexpression in colorectal adenocarcinoma might indicate that overexpression of the *WT1* gene is one of the critical genetic events in the tumorigenesis of colorectal adenocarcinoma. We previously reported that high expression levels of the *WT1* gene in myelodysplastic syndrome significantly correlated with disease progression to overt leukemia.[33] This raised the possibility that an increase in *WT1* expression levels reflected the disease progression of colorectal tumors from benign to carcinoma. Therefore, analysis of the correlation between the *WT1* gene expression levels and tumor progression stages, such as colorectal hyperplastic mucosa, adenoma, and carcinoma, is of interest and importance.

The present results are consistent with the idea that the wild-type *WT1* gene plays an important role in tumorigenesis of primary colorectal adenocarcinoma. Thus, WT1 could be a new molecular target for treatment of colorectal adenocarcinoma expressing WT1. We and others have demonstrated that WT1-specific cytotoxic T lymphocytes specifically kill WT1-expressing leukemia cells[34, 35] and that WT1 protein is an attractive tumor rejection antigen.[36–40] Our results presented here may provide a rationale for immunotherapy targeting WT1 protein as a new treatment strategy for colorectal adenocarcinoma expressing WT1.

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