
ORIGINAL RESEARCH

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Diagnostic and prognostic value of the methylation status of secreted frizzled-related protein 2 in colorectal cancer

Abstract

Purpose: The aim of this study was to investigate the diagnostic and prognostic significance of the methylation status of secreted frizzled-related protein 2 (*SFRP2*) in colorectal cancer (CRC).

Methods: Methylation-specific PCR assay was performed to analyze *SFRP2* promoter methylation in solid tissue, stool and serum samples collected from 169 CRC patients, 63 patients with advanced adenomas, 46 patients with non-adenomatous polyps and 30 normal healthy controls.

Results: Methylated *SFRP2* was frequently detected in CRC tissues and precancerous lesions. The sensitivity of *SFRP2* methylation levels in tissue, fecal and serum DNA for the detection of CRC was similar, ranging from 66.9 to 88.2%; however, serum *SFRP2* methylation levels showed a markedly higher specificity in discriminating CRCs from benign adenomas than those of *SFRP2* methylation levels in tumor and fecal DNA. Moreover, serum *SFRP2* methylation was significantly associated with poor differentiation grade ($P=0.019$), serosal/subserosal invasion ($P<0.001$), lymph node metastasis status ($P<0.001$) and TNM stage ($P<0.001$) of CRC. CRC patients with *SFRP2* hypermethylation in tumor, stool and serum samples had a significantly shorter overall survival than those negative for *SFRP2* methylation ($P=0.0216$, 0.0219 , and 0.0255 , respectively). Multivariate Cox regression analysis revealed that *SFRP2* promoter methylation in tumor samples was an independent prognostic factor for overall survival.

Conclusion: Our data suggest that serum *SFRP2* methylation status represents a promising, non-invasive marker for CRC detection and staging. Hypermethylated *SFRP2* may have prognostic relevance in patients with CRC.

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Colorectal cancer (CRC) is the third most common malignancy worldwide and the second leading cause of cancer-related death in Western countries [1,2]. This malignancy develops as a result of the transformation of normal colon epithelium to cancer via a stepwise histological progression sequence, proceeding from either adenomas or hyperplastic polyps/serrated adenomas [3,4]. Detection of early stage CRC and precancerous lesions has great promise to improve clinical outcomes and reduce mortality [5,6]. Yet, currently available non-invasive methods, such as fecal occult blood testing and measurement of serum carcinoembryonic antigen (CEA) levels, have low sensitivities and/or specificities for colorectal cancer [7-10]. Therefore, there is an urgent need to identify novel molecular markers in diagnosing and monitoring of this malignancy.

Epigenetic inactivation of tumor-related genes due to hypermethylation of CpG islands in the promoter region plays a key role in colorectal cancer carcinogenesis [3,11,12]. Examination of the aberrantly methylated gene promoter may thus be of value in distinguishing between malignant and benign tumors. Solid malignant tumors have been well documented to release significant amounts of DNA into the circulation system [13,14]. The tumor released DNA may represent a non-invasive biomarker for cancer detection. Indeed, assessment of aberrant DNA methylation in serum and fecal samples has been proposed as a promising high-throughput approach for the screening and monitoring of CRC [15-17].

Secreted frizzled-related proteins (SFRPs) comprise a family of secreted glycoproteins that function as inhibitive modulators of a putative tumorigenic pathway—the Wnt signaling pathway [18]. They contain an N-terminal cysteine-rich domain homologous to the putative Wnt-binding site of frizzled receptors, but lack a transmembraneous region as well as a C-terminal domain required for intracellular signal transduction [19]. The unique structure of SFRPs enables them to bind and sequester Wnt molecules from their cognate frizzled receptors, thus interfering with the Wnt pathway. Loss of SFRP2 expression due to promoter methylation has been frequently observed in many tumors, including colorectal, ovarian, breast, gastric and liver cancers [17,20-23]. Several earlier studies have demonstrated that the frequency of *SFRP2* promoter methylation in fecal DNA is markedly increased in CRC patients compared with normal healthy controls [24-25], suggesting the *SFRP2* hypermethylation in fecal DNA as a potential molecular biomarker of CRC. In this study, we extended the previous work and systematically evaluated the diagnostic potentials of *SFRP2* promoter methylation in tumor tissue, stool and serum

DNA samples in CRC. The prognostic value of *SFRP2* hypermethylation was also assessed in CRC patients.

Materials and Methods

Sample collection

The study was approved by the Ethical Committee of Yangzhou University (Yangzhou, China), and written informed consent was obtained from each study participant. Fresh tissue samples, including 169 cases of sporadic CRCs, 63 advanced adenomas, 46 hyperplastic polyps and 30 macroscopically normal colorectal mucosae, were collected at surgery or endoscopy from the First Affiliated Hospital of Yangzhou University. After removal, the tissue samples were immediately snap-frozen in liquid nitrogen and stored at -80°C . None of the sporadic CRC patients had received chemotherapy or radiation therapy prior to surgery. Haematoxylin and eosin staining was performed on all the samples for confirmation of diagnosis. CRC was staged according to the TNM classification system, and histological grade was assessed following the Broders' grading system. Advanced adenoma was defined as size ≥ 1 cm or having villous histology or high-grade dysplasia [26]. Stool samples were obtained from all participants after commencing bowel preparation the day before colonoscopy in a self-collection manner. Within 12 hours after collection, the samples were washed once with phosphate-buffered saline (PBS), centrifuged and stored at -80°C . Five milliliters of whole blood were withdrawn from each participant. Serum was obtained by centrifugation at $3000 \times g$ for 15 min at 4°C and stored at -80°C until analysis.

DNA extraction and methylation analysis

DNA was isolated from colonic tissues (5-10 mg), stool samples (250 mg), and serum samples (200 μl) using QIAamp DNA Mini Kit, QIAamp DNA Stool Mini Kit, and QIAamp Blood Mini Kit (Qiagen, Hilden, Germany), respectively, according to the manufacturer's protocols. Bisulfite treatment of the extracted DNA was performed using the EpiTect[®] Bisulfite Kit (Qiagen), which converts unmethylated cytosines to uracils while leaving methylated cytosines unaltered. The bisulfite-modified DNA was subjected to methylation-specific polymerase chain reaction (MS-PCR) for detection of the methylation status of the CpG-rich region (-219 to -81 relative to the transcription start site) in the *SFRP2* promoter, as described previously [27]. The aberrant methylation of this region is linked to transcriptional silencing of *SFRP2* in tumor cells. The MS-PCR primers are listed in Supplementary Table 1. Template-free distilled water was included as a negative control

TABLE 1. Frequencies of *SFRP2* methylation in tumor tissue, stool, and serum DNA of subjects with different colorectal diseases or normal healthy controls

Participant	Case (n)	M1 (%)	M2 (%)	M3 (%)
CRC	169	149 (88.2)	142 (84.0)	113 (66.9)
Adenoma	63	41 (65.1)	29 (46.0)	4 (6.4)
Non-adenomatous polyp	46	21 (45.7)	15 (32.6)	1 (2.2)
Normal control	30	0	2 (6.7)	0

M1, M2, and M3: *SFRP2* methylation in solid tissue, stool, and serum DNA samples, respectively.

TABLE 2. Sensitivity and specificity of *SFRP2* methylation in the diagnosis of colorectal cancer

Marker	Sensitivity ^a	Specificity ^b
M1	149/169 (88.2%)	22/63 (34.9%)
M2	142/169 (84.0%)	34/63 (54.0%)
M3	113/169 (66.9%)	59/63 (93.7%)

M1, M2, and M3: *SFRP2* methylation in solid tissue, stool, and serum DNA samples, respectively.

^aSensitivity was calculated as the number of cases with positive *SFRP2* methylation divided by the total number of cases with colorectal cancer (n=169).

^bSpecificity was calculated as the number of benign adenomas with negative *SFRP2* methylation divided by the total number of benign cases (n=63).

for the PCR. DNA from normal peripheral blood lymphocytes (PBL) was used as a control for the unmethylated *SFRP2* promoter, while *Sss* I methylase-treated DNA from PBL served as a positive control for the methylated *SFRP2* promoter. The success rate for the MS-PCR was approximately 99%. The PCR products were analyzed by electrophoresis in 2.5% agarose gels and stained with ethidium bromide.

Statistical analysis

Statistical analyses were performed using SPSS10.0 software (SPSS, Chicago, IL, USA). The correlation between *SFRP2* methylation and clinicopathologic features of CRC was analyzed with the χ^2 test. Overall survival was assessed using Kaplan-Meier curves with the log-rank test. Multivariate analysis, using a Cox proportional hazards model, was performed to identify prognostic factors associated with overall survival. *P* values of less than 0.05 were considered statistically significant.

Results

Analysis of SFRP2 methylation in solid tissue, stool and serum samples

The methylation status of *SFRP2* gene in DNA samples was determined from distinct solid tissue samples. The MS-PCR assay revealed that methylated *SFRP2* gene was most frequently detected in the CRC tissue specimens (149/169, 88.2%), followed by advanced adenoma (41/63, 65.1%) and non-adenomatous polyp (21/46, 45.7%) (Table 1). In contrast, none of the normal colorectal mucosas showed *SFRP2* hypermethylation (Table 1; Figure 1).

Next, the *SFRP2* methylation status in the stool and serum DNA samples was measured and compared with that observed in the corresponding solid tissue DNA samples (Figure 1). Similar to in the matched solid tissue DNA samples, *SFRP2* hypermethylation was frequently found in the fecal and serum DNA samples of CRC patients, although to a slightly lesser extent (Table 1). Patients with adenoma and non-adenomatous polyp showed a marked lower frequency of *SFRP2* hypermethylation in the fecal and particularly serum DNA samples.

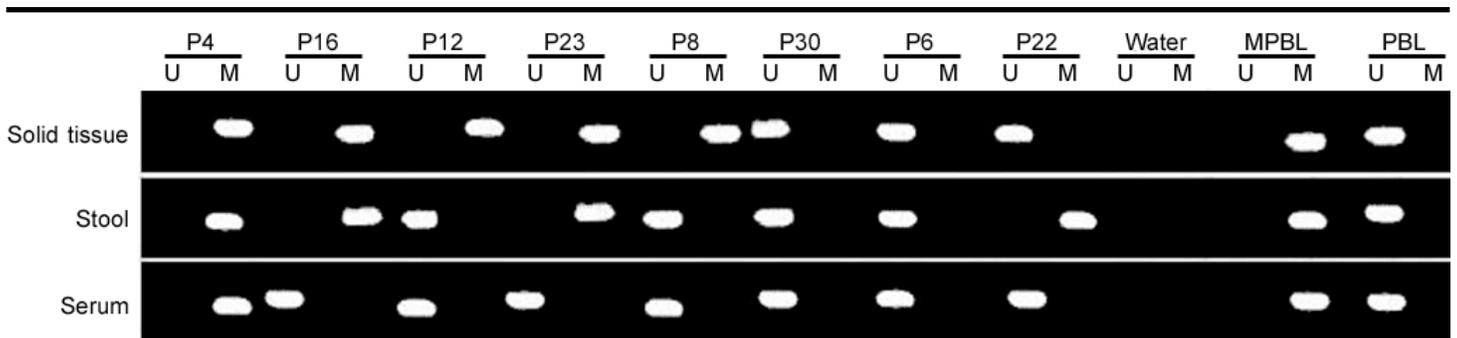


FIGURE 1. Assessment of *SFRP2* methylation status. Methylation-specific PCR was done on bisulfite-modified genomic DNA of solid tissue, stool and serum samples from representative cases with different colorectal diseases and healthy people. DNA from normal peripheral blood lymphocytes (PBL) was used as a control for the unmethylated *SFRP2* promoter (U), while *Sss* I methylase-treated DNA from PBL (MPBL) served as a positive control for the methylated *SFRP2* promoter (M). P4 and P16, participants with colorectal cancer; P12 and P23, participants with adenoma; P8 and P30, participants with non-adenomatous polyp; P6 and P22, normal controls.

TABLE 3. Correlation between *SFRP2* methylation status in tumor tissue, stool, and serum DNA of colorectal cancer patients and clinicopathological parameters

Parameter	Case (n)	M1 (%)	P	M2 (%)	P	M3 (%)	P
Age (year)			NS		NS		NS
<50	65	58 (89.2)		55 (84.6)		44 (67.7)	
≥50	104	91 (87.5)		87 (83.7)		69 (66.4)	
Gender			NS		NS		NS
Male	91	80 (87.9)		76 (83.5)		62 (68.1)	
Female	78	69 (88.5)		66 (84.6)		51 (65.4)	
Tumor location			NS		NS		NS
Ascending colon	32	28 (87.5)		26 (81.3)		22 (68.8)	
Transverse colon	20	17 (85.0)		16 (80.0)		11 (55.0)	
Descending colon	38	33 (86.8)		31 (81.6)		26 (68.4)	
Rectum	79	71 (89.9)		69 (87.3)		54 (68.4)	
Morphous			NS		NS		NS
Ulcer	69	61 (88.4)		58 (84.1)		47 (68.1)	
Lump	60	53 (88.3)		51 (85.0)		42 (70.0)	
Infiltrate	40	35 (87.5)		33 (82.5)		24 (60.0)	
Tumor size (cm)			NS		NS		NS
<5	114	103 (90.4)		98 (86.0)		73 (64.0)	
≥5	55	46 (83.6)		44 (80.0)		40 (72.7)	
Differentiation			0.026		0.039		0.019
Poorly	43	42 (97.7)		40 (93.0)		35 (81.4)	
Moderately	71	63 (88.7)		61 (85.9)		48 (67.6)	
Well	55	44 (80.0)		41 (74.6)		30 (54.6)	
Infiltration			0.002		0.003		<0.001
Submucosa/uscularis	64	50 (78.1)		47 (73.4)		22 (34.4)	
Serosa/subserosal	105	99 (94.3)		95 (90.5)		81 (77.1)	
Lymph node metastasis			NS		NS		<0.001
Positive	71	65 (91.6)		62 (87.3)		59 (83.1)	
Negative	98	84 (85.7)		80 (81.6)		44 (44.9)	
TNM stage			NS		NS		<0.001
I/II	99	86 (86.9)		82 (82.8)		53 (53.5)	
III/IV	70	63 (90.0)		60 (85.7)		60 (85.7)	

M1, M2, and M3: *SFRP2* methylation in solid tissue, stool, and serum DNA samples, respectively. NS, not statistically significant.

Diagnostic relevance of *SFRP2* methylation

To evaluate the diagnostic relevance of *SFRP2* methylation, we calculated its sensitivity (number of methylation-positive cancer cases/number of total cancer cases) and specificity (number of methylation-negative benign cases/total number of benign cases) in CRC. As shown in Table 2, the sensitivity for cancer detection using *SFRP2* methylation in tissue, fecal and serum DNA was similar, ranging from 66.9 to 88.2%. In contrast, the specificities in distinguishing CRCs from benign adenomas varied greatly, 34.9%, 54.0%, and 93.7% for *SFRP2* methylation

in tissue, fecal and serum DNA samples, respectively. These data revealed the potential diagnostic value of serum *SFRP2* methylation in CRC.

Correlation of *SFRP2* methylation with clinicopathologic parameters of CRC

The correlation between *SFRP2* methylation status and the clinicopathologic parameters of CRC was examined. The *SFRP2* methylation in tumor tissue was significantly correlated with poor differentiation grade ($P=0.026$) and serosal/

subserosal invasion ($P=0.002$) (Table 3); however, there was no correlation between *SFRP2* methylation status and other examined parameters including age, gender, tumor size, lymph node metastasis and TNM stage. Similar results were detected for this gene methylation in fecal DNA. Most interestingly, the serum *SFRP2* methylation was significantly associated with poor differentiation grade ($P=0.019$) and serosal/subserosal invasion ($P<0.001$), as well as with lymph node metastasis status ($P<0.001$) and TNM stage ($P<0.001$).

Prognostic significance of *SFRP2* methylation in CRC

The association of *SFRP2* methylation status with clinical outcome was analyzed in 77 CRC patients for whom follow-up information was available. The overall survival curves of the patients were shown in Figure 2. Patients with *SFRP2* hypermethylation in tumor, stool and serum samples had a significantly shorter overall survival than patients without *SFRP2* methylation ($P=0.0216$, 0.0219 and 0.0255, respectively; log-rank test). Univariate analysis showed that poor tumor differentiation ($P=0.013$), serosal/subserosal invasion ($P=0.012$), lymph node metastasis ($P<0.001$), advanced TNM stages ($P=0.033$), as well as *SFRP2* promoter methylation in tumor ($P=0.030$) and serum ($P=0.028$) DNA, were significant prognostic factors (Table 4). The multivariate analysis further revealed that the serosal/subserosal invasion, lymph node metastasis and *SFRP2* promoter methylation in tumor samples were independent prognostic factors (Table 4).

Discussion

Epigenetic silencing of tumor suppressor genes has been increasingly recognized as a causative mechanism in tumorigenesis. Accumulating evidence indicates that *SFRP2*, an important member of the SFRP family, functions as a negative regulator of the oncogenic Wnt pathway through competing with frizzled membrane-bound receptors [18,19]. In a broad range of malignancies including CRC, epigenetic inactivation of *SFRP2* by promoter methylation is frequently detected [17,20-23]. Here we demonstrated that *SFRP2* hypermethylation mostly frequently occurred in CRC patients, followed by patients with advanced adenomas and hyperplastic polyps. Normal controls, however, displayed an extremely low incidence of *SFRP2* methylation. It is noteworthy that hypermethylation of *SFRP2* gene was found in two endoscopically normal subjects, which was likely attributed to the presence of premalignant lesions. A previous study reported that the apparently normal mucosal field of patients with neoplasia had undergone epigenetic modification of a large number of genes, including *SFRP2*

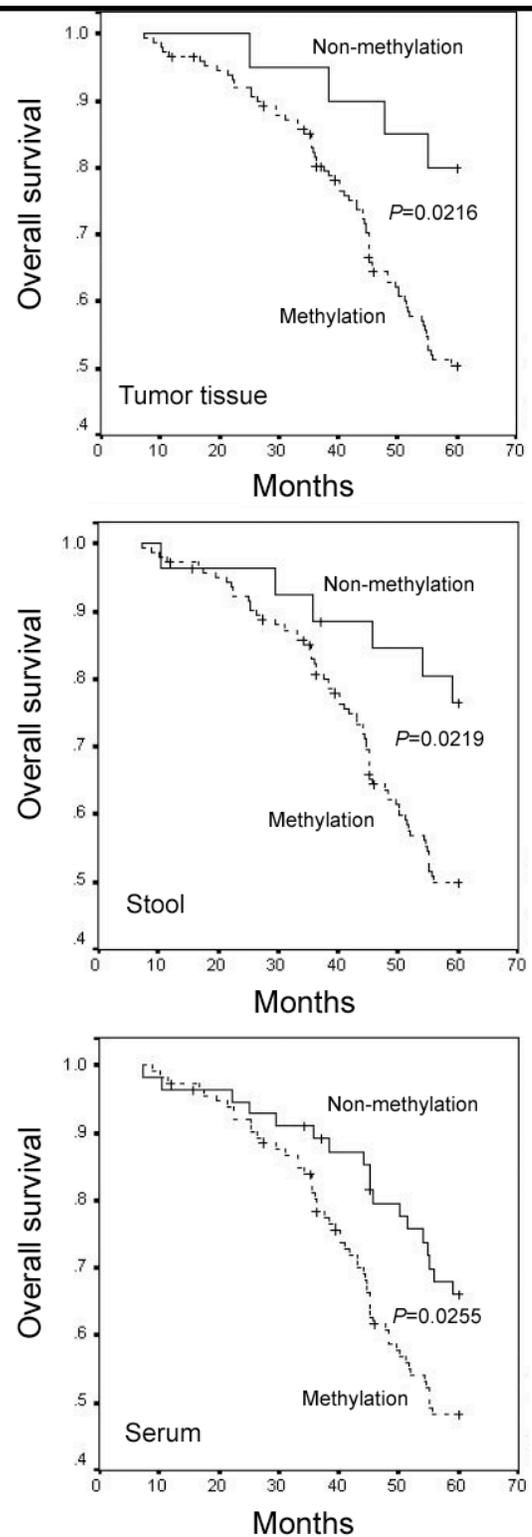


FIGURE 2. Overall survival in colorectal cancer patients according to the methylation status of *SFRP2* in tumor tissue, stool and serum DNA. Patients in the *SFRP2* methylation group showed a significantly worse survival than those in the non-methylation group.

TABLE 4. Univariate and multivariate analysis of prognostic factors for overall survival in patients with colorectal cancer

Univariate analysis	HR (95% CI)	P
Age (≥ 50 vs. < 50 years)	1.02 (0.64-1.63)	0.946
Gender (female vs. male)	0.98 (0.62-1.55)	0.935
Tumor size (≥ 5 vs. < 5 cm)	1.54 (0.96-2.48)	0.073
Tumor location		
Transverse vs. ascending colon	1.19 (0.51-2.78)	0.694
Descending vs. ascending colon	1.32 (0.67-2.63)	0.425
Rectum vs. ascending colon	0.83 (0.43-1.59)	0.573
Morphous		
Lump vs. ulcer	0.95 (0.56-1.60)	0.845
Infiltrate vs. ulcer	1.01 (0.56-1.82)	0.985
Differentiation		
Moderately vs. well	1.30 (0.74-2.28)	0.36
Poorly vs. well	2.14 (1.17-3.90)	0.013
Infiltration (serosa/subserosal vs. submucosa/uscularis)	1.93 (1.15-3.22)	0.012
Lymph node metastasis (positive vs. negative)	2.71 (1.71-4.30)	< 0.001
TNM stage (III/IV vs. I/II)	1.64 (1.04-2.60)	0.033
M1 (methylation vs. unmethylation)	3.06 (1.12-8.40)	0.030
M2 (methylation vs. unmethylation)	2.17 (1.00-4.74)	0.051
M3 (methylation vs. unmethylation)	1.81 (1.07-3.09)	0.028
Multivariate analysis	HR (95% CI)	P
Differentiation (poorly vs. well)	1.75 (0.89-3.43)	0.105
Infiltration (serosa/subserosal outside vs. submucosa/uscularis)	2.04 (1.17-3.58)	0.012
Lymph node metastasis (positive vs. negative)	2.79 (1.58-4.93)	< 0.001
TNM stage (III/IV vs. I/II)	1.37 (0.82-2.32)	0.231
M1 (methylation vs. unmethylation)	5.15 (1.07-24.81)	0.041
M2 (methylation vs. unmethylation)	1.02 (0.27-3.86)	0.972
M3 (methylation vs. unmethylation)	0.47 (0.21-1.08)	0.075

M1, M2, and M3: *SFRP2* methylation in solid tissue, stool, and serum DNA samples, respectively. HR, hazard ratio; 95% CI, 95% confidence interval.

[28]. These findings suggest that *SFRP2* methylation is closely linked to the initiation and progression of CRC carcinogenesis. Therefore, examination of *SFRP2* methylation status may provide useful information for detecting the early onset of CRC.

To further explore the clinical relevance of *SFRP2* methylation in CRC, the sensitivity and specificity of this potential diagnostic marker was evaluated. The results revealed similar high sensitivities for cancer detection using *SFRP2* methylation in tissue, fecal and serum DNA samples, ranging from 66.9 to 88.2%; however, the specificities in distinguishing CRCs from benign adenomas varied greatly, from ranging from 34.9% to 93.7%. Examination of the correlation between *SFRP2* methylation and the clinicopathologic parameters of CRC further revealed that *SFRP2* methylation in tumor tissue

and fecal DNA samples was significantly correlated with poor differentiation grade and serosal/subserosal invasion. Interestingly, serum *SFRP2* methylation seems more informative, which was found to be significantly associated not only with poor differentiation grade and serosal/subserosal invasion, but also with lymph node metastasis status and TNM stage. Hypermethylation of *SFRP2* in serum DNA was strongly correlated with shorter overall survival in CRC patients, as were the levels in tissue and fecal DNA. Moreover, *SFRP2* promoter methylation in tumor samples was an independent prognostic predictor of overall survival. Based on these findings in this cohort of patients, serum *SFRP2* hypermethylation appears to be a promising diagnostic and prognostic marker of CRC. Further investigation using a larger cohort of patients is needed to confirm this view.

SUPPLEMENTARY TABLE 1. Primer sequence, annealing temperature, and product size for MS-PCR assays

CpG status	Forward primer (5'-3')	Reverse primer (5'-3')	Annealing temperature (°C)	Product size (bp)
M	GGGTCGGAGTTTTTCGGAGTTGCGC	CCGCTCTCTTCGCTAAATACGACTCG	62	138
U	TTTTGGGTTGGAGTTTTTGGAGTTGTGT	AACCCACTCTCTTCACTAAATACAACCTCA	50	145

Note: M, methylated; U, unmethylated.

Previous studies have documented that DNA methylation of various genes in serum and plasma can be highly specific biomarkers for several human cancers [29,30]. Although the exact origins of this circulating cell-free DNA remain unknown, it has been proposed to be derived from apoptotic and necrotic cells [31]. In the present study, patients with precancerous lesions showed a significantly lower rate of *SFRP2* methylation in serum DNA than that in tissue DNA. In contrast, *SFRP2* methylation could be detected in serum DNA of patients with CRC at a similar incidence to that in tumor DNA. Moreover, serum *SFRP2* methylation was significantly correlated with lymph node metastasis status and TNM stage. Given these findings, we speculated that hypermethylated *SFRP2* in serum could reflect the nature of CRC, and with the progression of the disease, more tumor cells might acquire the ability to invade and metastasize through the lymphatic and blood system, thus leading to the increased production of methylated *SFRP2* from disseminated cancer cells.

In summary, our data highlight the diagnostic and prognostic value of *SFRP2* methylation in CRC. Hypermethylation of *SFRP2* in serum DNA can serve as a potential, non-invasive marker for early detection and staging of CRC. Hypermethylated *SFRP2* promoter may predict poor prognosis in patients with CRC.

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References

- Jemal A, Siegel R, Ward E. Cancer statistics, 2009. *CA Cancer J Clin* 2009; 59: 225-249.
- Ferlay J, Autier P, Boniol M. Estimates of the cancer incidence and mortality in Europe in 2006. *Ann Oncol* 2007; 18: 581-592.
- Grady WM, Markowitz SD. Genetic and epigenetic alterations in colon cancer. *Annu Rev Genomics Hum Genet* 2002; 3: 101-128.
- Jass JR, Young J, Leggett BA. Evolution of colorectal cancer: change of pace and change of direction. *J Gastroenterol Hepatol* 2002; 17: 17-26.
- Cappell MS. From colonic polyps to colon cancer: pathophysiology, clinical presentation, screening and colonoscopic therapy. *Minerva Gastroenterol Dietol* 2007; 53: 351-373.
- Winawer S, Fletcher R, Rex D, et al. Colorectal cancer screening and surveillance: clinical guidelines and rationale-Update based on new evidence. *Gastroenterology* 2003; 124: 544-560.
- Imperiale TF, Ransohoff DF, Itzkowitz SH, et al. Fecal DNA versus fecal occult blood for colorectal-cancer screening in an average-risk population. *N Engl J Med* 2004; 351: 2704-2714.
- Duffy MJ. Carcinoembryonic antigen as a marker for colorectal cancer: is it clinically useful? *Clin Chem* 2001; 47: 624-630.
- Ahlquist DA, Wieand HS, Moertel CG, et al. Accuracy of fecal occult blood screening for colorectal neoplasia. A prospective study using Hemoccult and HemoQuant tests. *JAMA* 1993; 269: 1262-1267.
- Greenberg PD, Bertario L, Gnauck R, et al. A prospective multi-center evaluation of new fecal occult blood tests in patients undergoing colonoscopy. *Am J Gastroenterol* 2000; 95: 1331-1338.
- Lee CK, Lee JH, Lee MG, et al. Epigenetic inactivation of the *NORE1* gene correlates with malignant progression of colorectal tumors. *BMC Cancer* 2010;10:577.
- Satelli A, Rao US. Galectin-1 is silenced by promoter hypermethylation and its re-expression induces apoptosis in human colorectal cancer cells. *Cancer Lett* 2011;301:38-46.
- van der Drift MA, Hol BE, Klaassen CH, et al. Circulating DNA is a non-invasive prognostic factor for survival in non-small cell lung cancer. *Lung Cancer* 2010;68:283-287.
- Czeiger D, Shaked G, Eini H, et al. Measurement of circulating cell-free DNA levels by a new simple fluorescent test in patients with primary colorectal cancer. *Am J Clin Pathol* 2011;135:264-270.
- Leung WK, To KF, Man EP. Quantitative detection of promoter hypermethylation in multiple genes in the serum of patients with colorectal cancer. *Am J Gastroenterol* 2005; 100: 2274-2279.
- Lenhard K, Bommer GT, Asutay S. Analysis of promoter methylation in stool: anovel method for the detection of colorectal cancer. *Clin Gastroenterol Hepatol* 2005; 3: 142-149.
- Muller HM, Oberwalder M, Fiegl H. Methylation changes in faecal DNA: a marker for colorectal cancer screening? *Lancet* 2004; 363: 1283-1285.

18. Jones SE, Jomary C. Secreted Frizzled-related proteins: searching for relationships and patterns. *Bioessays* 2002; 24: 811-820.
19. Uren A, Reichsman F, Anest V, *et al.* Secreted frizzled-related protein-1 binds directly to Wingless and is a biphasic modulator of Wnt signaling. *J Biol Chem* 2000; 275: 4374-4382.
20. Su HY, Lai HC, Lin YW, *et al.* An epigenetic marker panel for screening and prognostic prediction of ovarian cancer. *Int J Cancer* 2009; 124: 387-393.
21. Suzuki H, Toyota M, Carraway H, *et al.* Frequent epigenetic inactivation of Wnt antagonist genes in breast cancer. *Br J Cancer* 2008; 98: 1147-1156.
22. Nojima M, Suzuki H, Toyota M, *et al.* Frequent epigenetic inactivation of SFRP genes and constitutive activation of Wnt signaling in gastric cancer. *Oncogene* 2007; 26: 4699-4713.
23. Takagi H, Sasaki S, Suzuki H, *et al.* Frequent epigenetic inactivation of SFRP genes in hepatocellular carcinoma. *J Gastroenterol* 2008; 43: 378-389.
24. Huang ZH, Li LH, Yang F. Detection of aberrant methylation in fecal DNA as a molecular screening tool for colorectal cancer and precancerous lesions. *World J Gastroenterol* 2007; 13: 950-954.
25. Wang DR, Tang D. Hypermethylated SFRP2 gene in fecal DNA is a high potential biomarker for colorectal cancer noninvasive screening. *World J Gastroenterol* 2008; 14: 524-531.
26. Imperiale TF, Sox HC. Guidelines for surveillance intervals after polypectomy: coping with the evidence. *Ann Intern Med* 2008; 148: 477-479.
27. Cheng YY, Yu J, Wong YP, *et al.* Frequent epigenetic inactivation of secreted frizzled-related protein 2 (SFRP2) by promoter methylation in human gastric cancer. *Br J Cancer* 2007; 97: 895-901.
28. Belshaw NJ, Elliott GO, Foxall RJ, *et al.* Profiling CpG island field methylation in both morphologically normal and neoplastic human colonic mucosa. *Br J Cancer* 2008; 99: 136-142.
29. Laird PW. The power and the promise of DNA methylation markers. *Nat Rev Cancer* 2003; 3: 253-266.
30. Cottrell SE. Molecular diagnostic applications of DNA methylation technology. *Clin Biochem* 2004; 37: 595-604.
31. Jahr S, Hentze H, Englisch S, *et al.* DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells. *Cancer Res* 2001; 61: 1659-1665.