ORIGINAL ARTICLE

Analysis of *Syndecan-2* Methylation in Bowel Lavage Fluid for the Detection of Colorectal Neoplasm

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Background/Aims: *Syndecan-2* (*SDC2*) methylation was previously reported as a sensitive serologic biomarker for the early detection of colorectal cancer (CRC). The purpose of this study was to investigate whether *SDC2* methylation is detectable in precancerous lesions and to determine the feasibility of using *SDC2* methylation for the detection of CRC and precancerous lesions in bowel lavage fluid (BLF). **Methods:** A total of 190 BLF samples were collected from the rectum at the beginning of colonoscopy from patients with colorectal neoplasm and healthy normal individuals. Fourteen polypectomy specimens were obtained during colonoscopy. A bisulfite pyrosequencing assay and quantitative methylation-specific polymerase chain reaction were conducted to measure *SDC2* methylation in tissues and BLF DNA. **Results:** *SDC2* methylation was positive in 100% of villous adenoma (VA) and high-grade dysplasia, and hyperplastic polyp samples; 88.9% of tubular adenoma samples; and 0% of normal mucosa samples. In the BLF DNA test for *SDC2* methylation, the sensitivity for detecting CRC and VA was 80.0% and 64.7%, respectively, at a specificity of 88.9%. The BLF of patients with multiple tubular adenomas, single tubular adenoma and hyperplastic polyps showed 62.8%, 26.7% and 28.6% rates of methylation-positive *SDC2*, respectively. **Conclusions:** Our results demonstrated that *SDC2* methylation was a frequent event in precancerous lesions and showed high potential in BLF for detecting patients with colorectal neoplasm. **(Gut Liver 2018;12:508-515)**

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer globally and its incidence is rapidly increasing in South Korea, and it is one of main causes of cancer-related death.[1] In CRC, molecular level alterations occur before morphological alterations begin, and it progresses slowly through adenoma to dysplasia. Thus, it is possible to detect precancerous lesions by screening.[2] Early screening and detection of colorectal tumors can reduce CRC mortality. Despite the importance of a CRC screening test, the fact that only 40% of population over 50 years of age receive a colonoscopy examination indicates the need for improved practicability and accessibility of a screening test.[3]

The fecal occult blood test (FOBT) is the most widely-used noninvasive CRC screening test. It has been demonstrated to decrease CRC mortality in a randomized prospective study.[4] Yet, the test has limited sensitivity in detecting early stage CRC. The second-generation FOBT is an immunochemical test. It has improved sensitivity. Still, for advanced adenoma and early stage CRC, which are less likely to bleed, the diagnostic sensitivity is only around 20%. This has meant the continued use of the invasive colonoscopy examination to detect early stage colorectal tumor that can be completely cured by colonoscopic treatment. Colonoscopy is the gold standard for CRC diagnosis due to high sensitivity and specificity, whereas it is less preferred due to its invasiveness and difficulty of preparation. If it could be accurately predicted whether asymptomatic people harbor a tumor

or not by using a tumor specific biomarker aside from the FOBT, fear of CRC as well as unnecessary medical procedure can be reduced. Redundant medical procedures and their cost could be reduced if a noninvasive molecular diagnostic technology using characteristics of tumors, such as DNA methylation, could sensitively detect colorectal tumors instead of colonoscopy.

Harada et al.[5] reported that DNA methylation was detectable in bowel lavage fluid (BLF) collected from the patients with colorectal tumors during colonoscopy. BLF samples from invasive CRC revealed significantly higher levels of methylation than noninvasive tumors. BLF from CRC patients contained many exfoliated tumor cells and use of BLF could be an effective approach to detect CRC.[5,6]

Recently, *syndecan-2* (*SDC2*) has been identified as a novel potential epigenetic biomarker for the detection of CRC using a genome-wide Media CpG microarray approach. While *SDC2* methylation was detected at all CRC stages, even early stage showed very high frequency of *SDC2* methylation. Thus, it suggested that *SDC2* methylation can be potential biomarker for the detection of early stage of CRC.[7]

To date, *SDC2* methylation has not been studied in adenomas that are prodromal lesions of CRC. It is also necessary to confirm if *SDC2* methylation exfoliated from colon tumor can be detectable in BLF from patients with colon tumors before confirming detection in the stool.[5,7] Therefore, in this study, we performed bisulfite-pyrosequencing on an independent group of patients with precancerous lesions to confirm the high frequency of aberrant *SDC2* methylation in precancerous biopsies with various stages compared to normal tissues. For feasibility test of *SDC2* methylation in BLF samples for early CRC detection, we assessed *SDC2* methylation in patients with CRC and various precancerous lesions, and healthy individuals by quantitative methylation-specific polymerase chain reaction (PCR) assay. The results suggest that *SDC2* methylation test in BLF could be a useful biomarker for early CRC detection.

MATERIALS AND METHODS

1. Patients

Two hundred and eight patients agreed to participate in this study, those screened for CRC using a colonoscopy at Eulji General Hospital of Eulji University July 1, 2015 to May 30, 2016. Among them 18 patients were excluded due to incidentally combined disease such as infectious colitis or inflammatory bowel disease. A total of 190 patients were enrolled in this study. Before colonoscopy, patients were pretreated with 2 L of polyethylene glycol lavage solution and 10 mL of BLF specimens were collected from the rectum at the beginning of the colonoscopy. Based on colonoscopic and histologic findings, the BLF samples were divided into six groups: patients with CRC, villous adenoma (VA), multiple tubular adenomas (MTA), single tubular adenoma (STA), hyperplastic polyp (HP), and patients without colorectal lesions (N).

MTA were defined as being 1.0 cm or more in diameter, and more than three adenomas. STA was defined small adenomas less than three, and one tubular adenoma (TA) being 1.0 cm or more in diameter. The details of characteristics for enrolled patients are provided in Table 1.

2. Methods

1) Reagents

All chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise noted. Oligonucleotides and fluorescent probes were synthesized by Integrated DNA Technologies (Coralville, IA, USA).

2) Clinical specimens

Fourteen biopsy specimens were obtained from colorectal tumor during colonoscopy examination. Their identity as colorectal tumor was verified by an expert pathologist. Pathologic results were VA and/or high-grade dysplasia (n=2), TA (n=9), HP (n=3). Also five genomic DNA samples from normal mucosa without any history of malignancy were purchased from BioChain Institute (Hayward, CA, USA).

A total of 190 BLF samples from patients with CRC (n=10),

Table 1. Clinicopathologic Features of the Subjects in This Study

	Number	Male sex	Age, yr
Patients without colorectal lesion	54	15 (27.8)	56.6±13.6
Patients with hyperplastic polyp	21	10 (47.6)	55.7±12.1
Patients with single tubular adenoma	45	27 (60.0)	60.4±11.5
Patients with multiple tubular adenomas	43	32 (74.4)	64.4±10.4
Patients with villous adenoma and high-grade dysplasia	17	11 (64.7)	61.7±9.8
Patients with colorectal cancer	10	6 (60.0)	61.1±15.4
Total	190	101 (53.2)	59.9±12.3

Data are presented as number (%) or mean±SD.

VA and/or high-grade dysplasia (n=17), MTA (n=43), STA (n=45), HP (n=21) and N (n=54) were obtained by collecting 10 mL of remnant fluids in the rectum during insertion of the colonoscope. BLF were immediately frozen and stored at –80°C until used.

This study was approved by the Institutional Review Board of Eulji University (EMCS 2015-06-011-001). Written informed consent was obtained from all study participants, adhering to local ethics guidelines.

3) DNA isolation

Genomic DNA was isolated from tissue specimens using DNA Mini kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. BLF DNA was isolated using QIAamp DNA Stool Mini kit (Qiagen) according to manufacturer's instructions. The genomic DNA was finally eluted in 50 to 100 μL of TE buffer and frozen until use.

4) Bisulfite treatment

Genomic DNA was chemically modified using sodium bisulfite which converts all unmethylated cytosine to uracil, while leaving methylated cytosine unmodified, using the EZ DNA Methylation-Gold kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. In brief, genomic DNA was treated with sodium bisulfite for 2.5 hours at 65°C and desulfonation was performed for 20 minutes at room temperature. Bisulfite-converted DNA was purified using a Zymo-Spin IC column (Zymo Research) and eluted with 10 μL of distilled water. The eluted DNA was either used immediately for methylation analysis or was stored at –20°C until further use.

5) Quantitative bisulfite-pyrosequencing analysis

To quantify the methylation levels of *SDC2* in colorectal tissues, quantitative bisulfite-pyrosequencing[8] was performed. Specific bisulfite PCR and pyrosequencing primers were designed to analyze 149 bp of 5' untranslated region (UTR) including 4 CpG dinucleotides sites (+456, +460, +466, +473 bp) of *SDC2* gene using PyroMark Assay Design Software version 2.0 (Qiagen). Primer sequences were: forward, 5'-GGGAGTAGGAG-TAGGAGGAGGAA-3'; reverse, 5'- Biotin-ACCAAAACAAAAC-CAAACCTCCTACCCA-3'; sequencing, 5'-AGTAGGAGTAGGAG-GAGGAA-3'. Bisulfite-modified DNA (20 ng) was amplified in a 25 μL reaction volume with gene-specific primers using PyroMark PCR kit (Qiagen). Samples were heated to 94°C for 10 minutes and then amplified for 45 cycles at 94°C for 30 seconds, 61°C for 45 seconds and 72°C for 40 seconds, followed by a final extension at 72°C for 10 minutes. Pyrosequencing was performed using the PyroMark Gold Q96 reagent and PyroMark ID96 instrument (Qiagen) as instructed by the manufacturer. Briefly, 25 μL of each biotinylated PCR product was immobilized on streptavidin-coated Sepharose HP beads (Amersham Biosciences, Piscataway, NJ, USA) and sequenced using automatically generated nucleotide dispensation order for "sequence to analyze" corresponding to each reaction. Each CpG site was assigned a percentage (%) of methylation by evaluating the C to T ratio as methylation indexes (MtI). The average % of methylation across four CpG sites was obtained. Methylated non-CpG cytosines were used for internal controls, and to check the fidelity of bisulfite conversion. If MtI of each sample is greater than 5% of the detection limitation of pyrosequencing,[9] it considered as methylation-positive.

6) Two-step quantitative methylation-specific PCR

For measurement of *SDC2* methylation in BLF DNA, we used two-step fluorescence-based quantitative methylation-specific PCR (qMSP) method. For this assay, two methylation-specific primers and probe were designed to bind to bisulfite-converted methylated DNA for the 5' untranslated region (124 bp) of the *SDC2* gene by slight modification of primers described previously (Table 2).[7] To confirm PCR adequacy and quality of bisulfite-converted stool DNA, *COL2A1* (86 bp) was used as control gene (Table 2). In the first step of amplification, a total of 20 μL of reaction mixture contained 20 ng of bisulfite-converted stool DNA, reverse methylation-specific primer (0.05 μM) for *SDC2*, reverse specific primer (0.05 μM) for control *COL2A1* and 4 μL of 5× AptaTaq PCR master mix (Roche Diagnostics, Basel, Swiss). Linear amplification was performed as follows: one cycle at 95°C for 5 minutes and 35 cycles at 95°C for 15 seconds and

Table 2. Sequences of Primers and Probes Used in Two-Step qMSP

Gene	Primers or probes	Sequences (5'→3')
SDC2	Forward*	GTAGAAATTAATAAGTGAGAGGG_C_
	Reverse*	A_CG_ACTCAAACT_CG_AAAACT_CG_
	Probe*	FAM-TT_CG_GGGG_CG_TAGTTG_C_GGG_CG_G-Iowa Black
COL2A1	Forward†	GTAATGTTAGGAGTATTTTGTGGITA
	Reverse†	CTAICCCAAAAAAACCCAATCCTA
	Probe	FAM-AGAAGAAGGGAGGGGTGTTAGGAGAGG-Iowa Black

qMSP, quantitative methylation-specific polymerase chain reaction.
*Underlining indicates CpG nucleotides; †I represents inosine nucleotide.
Adapted from Oh T, *et al.* J Mol Diagn 2013;15:498-507.[7]

at 60°C for 60 seconds.

After the first step amplification, reaction mixture was divided into two portions and each 16 μL and 4 μL was used for *SDC2* and *COL2A1* amplification, respectively. For *SDC2* amplification, a total of 40 μL reaction mixture contained each 0.25 μM of methylation-specific forward and reverse primers, 0.125 μM of fluorescent probes (FAM) and 8 μL of 5× PCR master mix. A total of 40 μL reaction mixture for *COL2A1* amplification was contained each 0.25 μM of gene-specific forward and reverse primers, 0.1 μM of fluorescent probes (FAM) and 8 μL of 5× AptaTaq PCR master mix (Roche). qMSP was performed on a Rotor-Gene Q real time PCR system (Qiagen, Hilden Germany). The thermal cycling conditions were as follows: one cycle at 95°C for 5 minutes and 40 cycles at 95°C for 15 seconds and at 60°C for 60 seconds. All reactions were performed in duplicate. For each run, methylated DNA (Qiagen, Hilden, Germany) and unmethylated DNA (Qiagen, Hilden, Germany) were used as methylation controls. Nontemplate control was also included. The C_T (cycle threshold) value was calculated by using Rotor Gene Q software.

The *SDC2* methylation results for individual samples were scored as C_T value, as detected by the instrument software. A sample in *SDC2* methylation test was considered as positive if the value of *SDC2* C_T was less than 40 cycles when *COL2A1* C_T was less than 36 cycles. It was considered as negative if the C_T of *SDC2* was not detected when *COL2A1* C_T was less than 36 cycles. Stool samples from patients were classified as positive if one out of two PCR replicates were called methylation-positive and as negative if none of two PCR replicates called methylation-negative (1 out of 2 algorithm).

7) Statistical analysis

All statistical analysis was performed using MedCalc version 9.3.2.0 (MedCalc Software, Ostend, Belgium). A p-value <0.05 was considered statistically significant. Receiver operating characteristic (ROC), area under ROC (AUC) and 95% confidence intervals (CIs) were calculated.
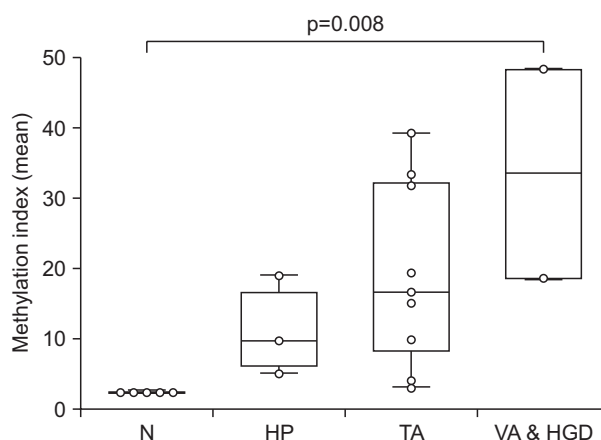
Fig. 1. Methylation assessment of *SDC2* gene in colorectal tissues by bisulfite pyrosequencing. The methylation level of the *SDC2* gene was evaluated in normal mucosa (N), hyperplastic polyp (HP), tubular adenoma (TA) and villous adenoma and high-grade dysplasia tissues (VA & HGD). The methylation indexes (MtIs) of each sample are represented with box-and-whisker plots. The difference in the MtI of *SDC2* was statistically significant at p<0.01 in VA & HGD vs TA vs HP vs N by the Kruskal-Wallis test.
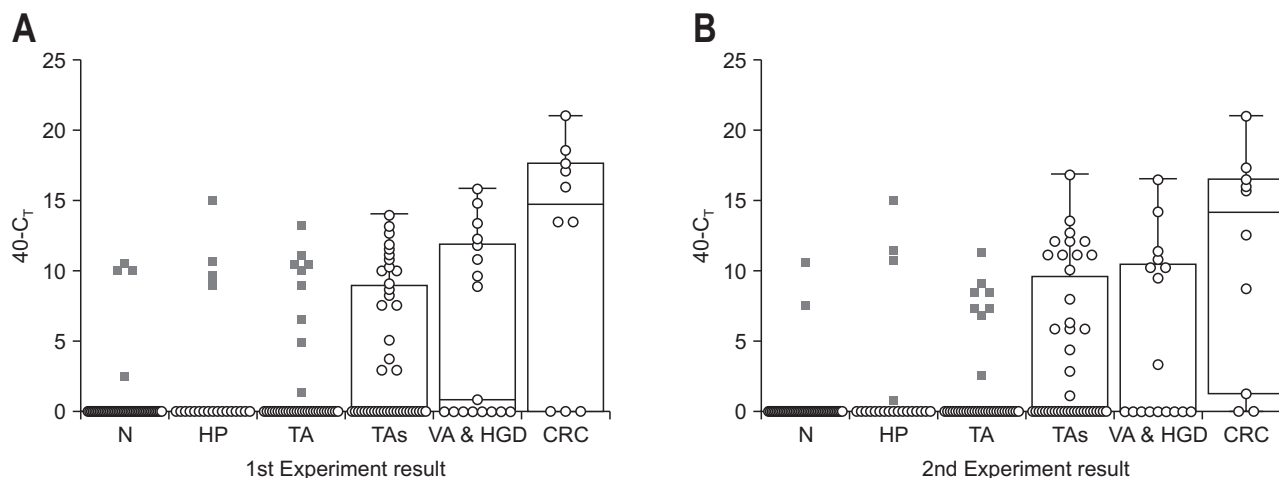
Fig. 2. Results of *SDC2* methylation analysis in bowel lavage fluid by two-step quantitative methylation-specific polymerase chain reaction (qMSP) test. (A, B) Two-step qMSP was performed twice in DNA from bowel lavage fluid. The distribution of *SDC2* methylation was expressed in C_T (threshold cycle) values as 40-C_T for each sample. The methylation status of the *SDC2* gene is shown as box-and-whisker plots. N, normal colonoscopy; HP, hyperplastic polyps; TA, single tubular adenoma; TAs, multiple tubular adenomas; VA & HGD, villous adenoma and/or high-grade dysplasia; CRC, colorectal cancer.

RESULTS

1. *SDC2* methylation in tissue samples from patients with colorectal neoplasia

Previous study has reported that aberrant methylation of *SDC2* is not observed in normal mucosa tissues, while frequently detected in colorectal tumor tissues.[6] Presently, *SDC2* methylation in precancerous lesions was examined using bisulfite-pyrosequencing in tissue specimens from VA or high-grade dysplasia (AA, n=2), TA (n=9), HP (n=3) and normal mucosa (N, n=5). *SDC2* methylation was positive in 100% of AA, 88.9% of TA and 100% of HP tissue, and was negative in all of normal mucosa. The level of *SDC2* methylation was significantly increased according to the malignant potential of precancerous lesions (p=0.008) (Fig. 1).

2. *SDC2* methylation in bowel lavage fluid from patients with colorectal neoplasia

To determine whether *SDC2* methylation can detect CRC and precancerous lesions, two-step qMSP was performed twice in BLF from patients with CRC (n=10), VA & HGD (n=17), TAs (n=43), TA (n=45), HP (n=21) and N (n=54) (Fig. 2). The degree of *SDC2* methylation in each sample was determined as a C_T by qMSP and expressed as $40\text{-}C_T$. A higher $40\text{-}C_T$ value represents a higher methylation level of *SDC2* and represented as 0, if C_T of *SDC2* was undetectable.

Overall methylation positivity was gradually increased depending on severity of lesion. ROC analysis was conducted to measure sensitivity and specificity of *SDC2* methylation for diagnosis of CRC and VA & HGD using 1 out of 2 algorithm (Fig. 3). Sensitivities for detecting CRC and VA & HGD were
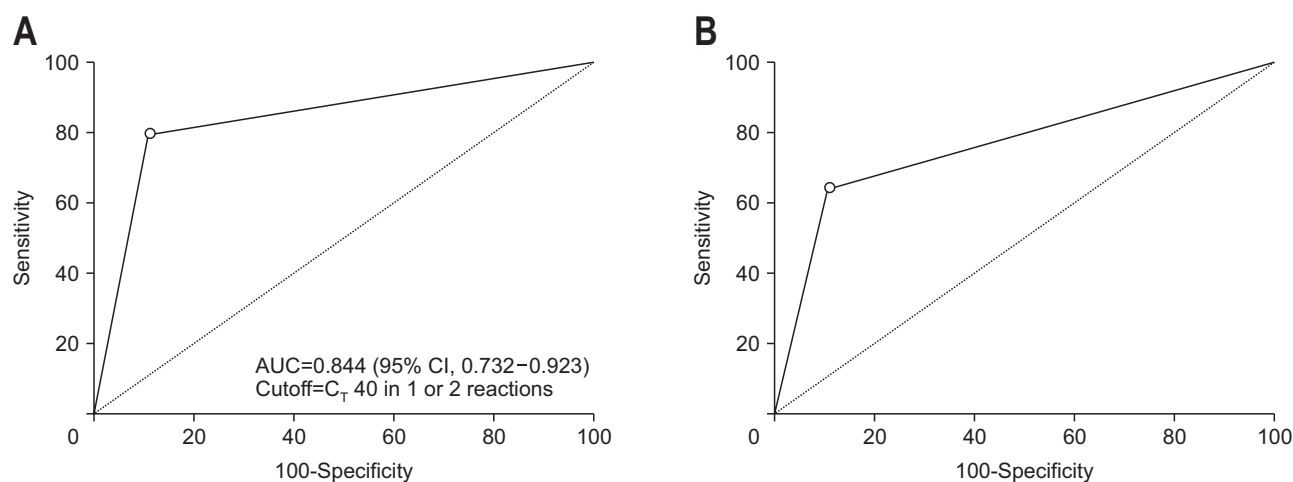
AUC=0.844 (95% CI, 0.732−0.923)
Cutoff=C_T 40 in 1 or 2 reactions

Fig. 3. Receiver operating characteristic (ROC) for *SDC2* in detecting colorectal cancer (CRC). ROC curves were plotted as CRC vs healthy normal controls (A) and VA & HGD vs healthy normal controls (B). The cutoff values for methylation-positive, p-values, area under ROC (95% confidence interval [CI]), sensitivity (95% CI) and specificity (95% CI) are indicated at the bottom.
AUC, area under curve; VA & HGD, villous adenoma and/or high-grade dysplasia.

Table 3. Methylation Positivity of *SDC2* in Bowel Lavage Fluids from Patients with Colorectal Neoplasia

Algorithm	Methylation positivity					
	N	HP	TA	TAs	VA & HGD	CRC
1 out of 2	11.1 (6/54)	28.6 (6/21)	26.7 (12/45)	62.8 (27/43)	64.7 (11/17)	80.0 (8/10)

Data are presented as percent (number/number).
N, normal colonoscopy; HP, hyperplastic polyp; TA, single tubular adenoma; TAs, multiple tubular adenomas; VA & HGD, villous adenoma and/or high-grade dysplasia; CRC, colorectal cancer.

Table 4. Methylation Positivity of *SDC2* in Bowel Lavage Fluid According to the Number of Detected Adenomas

No. of adenoma	1	2–5	>5	p-value*
Methylation positivity	26.7 (12/45)	57.9 (22/38)	100 (5/5)	<0.01

Data are presented as percent (number/number).
*Calculated by linear-by-linear association test.

80.0% (8/10; 95% CI, 44.4% to 96.9%) and 64.7% (11/17; 95% CI, 38.4% to 85.7%), respectively with a specificity of 88.9% (6/54; 95% CI, 77.4% to 95.8%). Methylation positivity for MTA was 62.8% (27/43), significantly higher than that of STA 26.7% (12/45). Interestingly, HP also showed the methylation positivity of 28.6% (6/21) (Table 3). These results suggest that *SDC2* methylation can be detected in patients with precancerous lesion and had a great role in CRC prevention as a time indicator for colonoscopic polypectomy of precancerous lesions.

SDC2 methylation positivity was also significantly increased depending on the number of detected adenomas (p<0.01, linear-by-linear association test). Positivity was 26.7% (12/45) for a single adenoma, 57.9% (22/38) for 2–5 adenomas and 100% (5/5) for 6 or more adenomas (Table 4).

DISCUSSION

The results of this study demonstrated that *SDC2* methylation was frequently occurred in precancerous lesions in tissues and BLF samples. The incidence of CRC is rapidly increasing worldwide,[1] and the development of screening has increased the survival rate of CRC over the past 20 years. A FOBT, computed tomography (CT) colonography, double-contrast barium enema, flexible sigmoidoscopy, and colonoscopy are commonly used to screen CRC.[10] However, the sensitivity and specificity of the FOBT and barium enema for CRC are low,[11-15] and a CT colonoscopy has a limitation with CT-related radiation exposure. Flexible sigmoidoscopy cannot explore the entire colon, and a screening colonoscopy is invasive and can be vary by the patients' status and doctor's ability. Recent research suggests that CRC may be detectable using DNA markers.[4,6,8,9] Colorectal epithelial cells are continuously exfoliated, usually more often in colorectal tumors than in normal tissue. Validation of this method as a screening test for colorectal tumors has been explored.[16] Other studies have sought genetic and epigenetic biomarkers for CRC detection.[17,18] DNA mutations including K-ras, p53, adenomatous polyposis coli (APC), microsatellite instability (MSI) markers and unfragmented L-DNA have been used as genetic biomarkers for CRC diagnosis.[19-23] However, a large-scale prospective study using 21 combinations of genetic marker (K-ras, APC, p53, MSI, BAT and L-DNA) in stool reported a diagnostic sensitivity of CRC and colorectal adenoma of 52% and 41%, respectively, which was superior to the 13% and 14% respective sensitivity of FOBT.[24] These studies imply that the DNA panel has better sensitivity and adequate specificity in colorectal tumor diagnosis compared to the FOBT. However, the performance is not satisfactory considering its cost.

The limitation of genetic biomarkers has prompted studies using epigenetic biomarkers. Approximately 60% of human genes have CpG islands, where cytosine and guanine are concentrated at their promoters. Epigenetic biomarkers refer to hypermethylation in the promoter region, which is observed early in the development of human cancer. This gene methylation marker has been used in attempts to improve the diagnostic sensitivity of CRC and advanced adenoma.[25,26] Typical epigenetic markers are *SFRP2*, CpG island methylator phenotype, intestinal stem cell marker DCLK1 and SEPT9. Sensitivity of CRC increased by over 90%, but adenoma sensitivity and specificity were both was 70%, which is a limitation to use as a general CRC test.[27-32]

The clinical trial of multitarget stool DNA testing as a CRC screening test compared to the FOBT has been explored.[31] The DNA test included the *KRAS* mutations along with *NDRG4* and *BMP3* methylations, which are epigenetic markers. The study represented sensitivity of 92.3% for the DNA test and 73.8% for fecal immunochemical test (FIT) in detecting CRC. For those with advanced precancerous lesions, sensitivities of the DNA test and FOBT were only 42.4% and 23.8%.[33] Among patients in a subclinical stage who were at average risk for CRC, multitarget stool DNA testing had higher sensitivity compared to FIT, while the false positive rate was still significantly high. These prior studies suggested that the epigenetic biomarkers were still unsatisfactory and need for more sensitive and specific epigenetic biomarkers.

Syndecan-2 is a cell membrane protein that is involved in cell proliferation, cell migration and in the interaction between cells and intercellular substances. *SDC2* is not expressed in normal intestinal endothelial cells, but is expressed in mesenchymal cells.[34] It was previously reported that *SDC2* was aberrantly methylated in tumor tissues of most CRC patients and demonstrated a high potential of quantification of *SDC2* methylation in blood for early detection of CRC.[7] *SDC2* methylation has been described in more than 95% of CRC tissue and no difference with respect to the CRC stage has been found.[7] This implies that *SDC2* methylation may begin early in tumor development and continue thereafter.

Up to date, *SDC2* methylation has not been addressed in precancerous lesions, thus this study was conducted to confirm the *SDC2* methylation in precancerous biopsies at various stages. It is new that *SDC2* methylation appears 100% in the stage of advanced adenoma and 88.9% of non-advanced adenomas, while did not occur in normal tissues. Thus it provides good evidence for *SDC2* methylation as a potential biomarker for the early detection of CRC and prevention to CRC development.

The usefulness of *SDC2* as a CRC biomarker for the first time was studied from blood samples and showed high sensitivity and specificity in the serum of CRC patients, and almost no methylation in serum DNA of normal healthy population, that resulted in suggesting the possibility as a circulating biomarker.[7] If these epigenetic markers can detect tumor specific DNA methylation separated from the tumor at stool before being detected in the serum, the epigenetic marker can be noninvasive and more sensitive biomarkers in CRC screening. Thus, we tried to confirm *SDC2* methylation in BLF before confirming the detection in the stool.

In this study, *SCD2* methylation test by qMSP in BLF showed sensitivities of 80.0%, 64.7% and 62.8% for CRC, VA and MTA, respectively at the specificity of 88.9%. Meanwhile, Harada *et al.*[5] analyzed the methylation of 15 genes in BLF of patients with CRC and advanced adenoma and identified three highly sensitive genes. Sensitivities of *miR-124-3*, *LOC386758*, and *SFRP1* were 71.8%, 79.5% and 4.4%, respectively, for CRC detection. Combination of three genes revealed 82% of sensitivity and 79% of specificity with the AUC of 0.834, which are comparable to our data.

The *SDC2* methylation in the BLF displayed high sensitivity and specificity as a marker for the detection of CRC patients. Also, it is encouraging that sensitivity and specificity increased to the expected levels of clinically important precancerous lesion such as VA and/or high-grade dysplasia. Sensitivity of *SDC2* methylation significantly increased with more than three adenomas than 1 or 2 adenomas. The false positive rate in the normal group was 11.1% and positive rate in STA or HP was less than 30%. This is an acceptable limitation for the use of single biomarker in BLF. Thus, a large scale of clinical validation will be warranted in voided stool samples to evaluate the clinical utility of stool-based *SDC2* methylation test to early detect CRC.

In conclusion, the *SDC2* DNA methylation was detected in the tissue of almost colorectal neoplasm. The *SDC2* DNA methylation in BLF showed a high sensitivity and specificity in patients with CRC and precancerous lesions. Also it has potential value in detection of the patients with precancerous lesion.

CONFLICTS OF INTEREST

S.A. and T.J.O. are employees and shareholders of Genomictree, Inc. The other authors declare that they have no conflicting interests.

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