*Article*

# A novel stool-based *SDC2* DNA methylation test is more robust than FIT and plasma CEA in detecting colorectal neoplasia in China

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## ABSTRACT

**BACKGROUND: Recent studies have shown that aberrant methylation of *Syndecan-2* (*SDC2*) can be detected in stool samples from patients with colorectal cancer (CRC) and advanced adenoma (AA). This study aims to evaluate both the effectiveness and accuracy of a stool DNA (sDNA) test of methylated *SDC2* in detecting CRC and AA in comparison to a fecal immunochemistry test (FIT) and plasma carcinoembryonic antigen (CEA). METHODS: The study enrolled 120 participants who were either diagnosed with CRC and AA or were CRC- or AA-negative according to colonoscopy. Stool samples for each participant were collected and subjected to both FIT and the sDNA test for *SDC*2 methylation. Meanwhile, peripheral blood was drawn to assess plasma CEA level. Analytical performance of these three detection methods was compared in terms of sensitivity and specificity. Furthermore, Spearman correlation was performed to evaluate possible correlation between *SDC2* methylation and clinical characteristics in CRC patients. RESULTS: The sensitivity of the sDNA test in**

**detecting CRC was 90.7%, significantly higher than both FIT (58.1%) and plasma CEA (20.9%). The detection rate of the sDNA test for AA was also respectable at 52.7%, dramatically better than FIT (9.1%) and plasma CEA (3.6%). The sDNA test was also particularly robust for stage I/II CRC at 87% sensitivity. Moreover, it could detect a significant number of colonic carcinomas *in situ* as well as high-grade intraepithelial neoplasia, besting FIT and CEA by a large margin (11/13 vs. 3/13 vs. 1/13). For both SDC2 testing and FIT, the specificity was 86.4%, lower than the 90.9% specificity observed for plasma CEA. Interestingly, we found that *SDC2* methylation status was positively correlated with tumor location, TNM staging, and size, but inversely correlated with age at diagnosis. CONCLUSIONS: Single-target *SDC2* methylation testing is a more sensitive detection strategy for CRC and AA than conventional approaches such as FIT and plasma CEA in China. (Am J Transl Med 2020. 5(1):37-50).**

**Keywords:** Colorectal neoplasia, *SDC2*, Stool DNA, Gene methylation, Sensitivity
(Manuscript received July 5, 2020, accepted July 28, 2020; published online January 30, 2021)

# INTRODUCTION

Colorectal cancer (CRC) is the third most common malignant tumor and the second leading cause of cancer-related death worldwide (Bray et al., 2018). In China, CRC is the fourth most common cause of death for women and the fifth for men. Moreover, the morbidity and mortality rates of CRC have risen in recent decades (Chen et al., 2016). Since the 5-year survival rate of CRC is closely related to the stage of the malignancy (Cellini et al., 2020; Jemal et al., 2017), early detection and preventive measures are essential to reduce the incidence and mortality of CRC (Bray et al., 2018). Micro-simulation modeling shows that the decline of the CRC death rate is consistent with increased screening (Knudsen et al., 2016), an important measure to detect precancerous lesions or new-onset CRC in the asymptomatic phase (Levin et al., 2008). Several countries have developed national screening programs to reduce the incidence of CRC (Imperiale et al., 2014; Rex et al., 2017b; Zhang et al., 2015). Among them, routine methods for screening the average-risk population include fecal occult blood test (FOBT), fecal immunochemistry test (FIT), plasma carcinoembryonic antigen (CEA), and colonic endoscopy. Though these methods show benefits in detecting CRC, each has various drawbacks including a subpar performance–price ratio, limited accuracy, or invasiveness. Therefore, mass screening for CRC in China demands a more effective screening method in order to be genuinely effective.

In recent years, stool-based testing has attracted widespread attention due to its noninvasiveness and elimination of the need for extensive bowel preparation. A multitarget stool DNA (sDNA) test demonstrated excellent performance characteristics in a large-scale CRC screening in the United States, showing 92.3% sensitivity and 86.6% specificity (Imperiale et al., 2014; Lai et al, 2018). The test detects CRC-associated alterations in several genes – including a somatic mutation in *KRAS* and promoter hypermethylation in *BMP3* and *NDRG4* – in addition to hemoglobin by FIT. The high-throughput and quantitative fecal DNA detection technology was later improved to increase sensitivity for precancerous lesions and adenomas (Ahlquist et al., 2012). In 2014, it was approved for commercial use by the US Food and Drug Administration (FDA), and subsequently recommended as a novel CRC screening option by the US Preventive Services Task Force, the US Multi-Society Task Force, and the National Comprehensive Cancer Network (Bibbins-Domingo et al., 2016; Provenzale et al., 2018; Rex et

al., 2017a; Wolf et al., 2018). However, this test is more complex and expensive than other methods such as FIT and plasma CEA.

The development and progression of CRC, from adenomas to adenocarcinomas, is associated with abnormal methylation of a wide spectrum of genes (Barták and Kalmár, 2017; Mitchell et al., 2014; Oh et al., 2013; Okugawa et al., 2015; Ørntoft et al., 2015; Park et al., 2018; Yang et al., 2013). In recent years, the *SDC2* gene has been found to be hypermethylated in CRC cell lines and tissues, but hypomethylated in normal tissues (Choi et al., 2015; Jang et al., 2017; Kim and Park, 2018; Mitchell et al., 2014; Niu et al., 2017; Oh et al., 2013; Oh et al., 2017; Park et al., 2018). *SDC2* encodes a highly glycosylated membrane protein that is a member of the synaptoprotein family of heparin sulfate proteoglycans. As a co-receptor for cell signaling and for extracellular matrix molecules, *SDC2* plays an indispensable role in cell-cell adhesion and communication (Essner et al., 2006). In tumorigenesis and metastasis, *SDC2* is also essential in regulating adhesion, migration, angiogenesis, and metastasis (Choi et al., 2017; Sun et al., 2014). Therefore, it provides a potential target for developing molecular markers and therapeutic agents to detect and treat CRC (Choi et al., 2015; Jang et al., 2017; Mytilinaiou et al., 2017). Recently, *SDC2* promoter hypermethylation was found capable of discriminating normal individuals from people with CRC and large adenomas in both tissue and stool specimens (Niu et al., 2017). Therefore, a single-target sDNA test of methylated *SDC2* was subsequently developed (Wang et al, 2020).

The current study compared the performance of a sDNA test of methylated *SDC2* to FIT and plasma CEA for the detection of CRC in a hospital-based cohort of 120 participants. The sensitivity and specificity of the test were computed and compared with those of FIT and plasma CEA, and the correlation between SDC2 methylation and several clinical features of CRC was also investigated, providing new data to help evaluate and select appropriate CRC screening strategies.

# SUBJECTS AND METHODS

## Study participants

The study enrolled 155 individuals of Han Chinese ancestry from the Digestive Center of Songjiang Hospital from April 13, 2018 to July 30, 2019. Participants who met any of the following criteria were included: (i) Subjects who underwent colonoscopies and, in case of abnormal findings, had biopsies and were diagnosed based on the pathological reports; (ii) Patients with CRC who had been diagnosed prior to surgery; (iii) Patients whose pathological findings were benign or malignant before endoscopic resection of adenomas; (iv) Patients whose stool samples were collected before bowel preparation prior to colonoscopy or at least one week after colonoscopy but before surgeries to remove CRC and advanced adenoma (AA). All medical personnel involved in CRC testing (including the *SDC2* methylation test, FIT, and plasma CEA) were blinded to the participants' diagnostic status. All tests for CRC or AA patients, who were nonrandomly selected for the current clinical study, were conducted before the cancerous or precancerous lesions were removed via surgical or endoscopic resection. AA was defined as adenoma with either maximum diameter $\geqslant$1 cm, high-grade intraepithelial neoplasia, or substantial villous structures (Imperiale et al., 2014). Stool and plasma samples from control individuals were used to minimize analytical bias and assess false-positive rates in this outpatient setting. Some patients were

excluded from the study based on the following criteria: (i) Patients with other malignant tumors; (ii) Patients with CRC who had received radiotherapy and/or chemotherapy; (iii) Patients without colonoscopy biopsy or postoperative pathology information; (iv) Patients with ambiguous diagnosis; (v) Patients with loose stools, watery stools, or inability to provide stool samples; (vi) Patients whose stool samples were collected less than one week after colonoscopy but before surgery for CRC and AA; (vii) Patients with neuroendocrine neoplasia; (viii) Cases with incomplete information on FIT and plasma CEA. Based on these criteria, a total of 35 enrolled subjects were excluded from the final analysis (22.6%). Our study was performed in accordance with the principles of the Declaration of Helsinki with regard to ethical research involving human subjects, and the protocols were approved by the Institutional Review Board of Songjiang Hospital (approval ID: 201811). Written informed consent was obtained from all participants prior to enrollment.

## Fecal sample collection and processing

Fecal samples with an average weight of 4.5 g were collected from each participant using a semi-quantitative stool collection device (Creative Biosciences, Guangzhou, China). Samples were kept in a preservation buffer to prevent DNA degradation. The buffer-protected stool samples were transported to the designated testing laboratory within 3 days. The stool samples were homogenized and centrifuged immediately after they were received by the lab, and the supernatants were aliquoted and stored at −80°C for subsequent processing.

## DNA extraction and bisulfite treatment

Target human genes in stool DNA including *SDC2* and the reference gene *β-actin* (ACTB) were purified and detected with a methylation-specific detection kit (Creative Biosciences, Guangzhou, China). Briefly, 3.2 ml crude stool samples were placed in a filter tube and centrifuged at 5,000 rpm for 5 min at room temperature. The supernatant was incubated with 2 ml lysis buffer and 50 µl M1 magnetic beads at 95°C for 15 min and cooled at room temperature for 1 h. The formed bead/hybrid complexes were washed with 800 µl washing buffer, denatured in 50 µl sodium hydroxide solution, and treated with 100 µl sulfite buffer at 65°C for 70 min. The captured DNA was mixed with M2 magnetic beads in binding buffer and incubated for 15 min at room temperature. After two washes with 800 µl washing buffer and treatment with 200 µl desulfonic acid solution for 15 min at room temperature, the bisulfite-treated DNA samples were again washed twice and collected in 60 µl elution buffer.

## Quantitative methylation-specific PCR (qMSP)

Real-time qualitative methylation-specific PCR (qMSP) was performed to detect *SDC2* methylation in DNA samples. ACTB was used as a reference gene for bisulfite treatment and DNA input. PCR amplification was run on LightCycler 480II (Roche, Basel, Switzerland) under the following cycling conditions: 95°C for 5 min, 48 cycles at 95°C for 20 s, 58°C for 60 s, and 72°C for 30 s, and a final cooling step at 37°C for 30 s. For every run, the bisulfite-treated DNA samples were amplified together with a water blank, a positive control, and a negative control. The complementary strand to the TaqMan probe of methylated *SDC2* was used to calculate marker performance.

## Results interpretation

The cycle threshold (Ct) value of *SDC2* methylation was computed by an Abs Quant/2nd Derivative Max method on Roche Light Cycler 480II. Patient samples

were considered valid if the Ct value of *ACTB* was ⩽36. The results were dichotomized into positive and negative based on a pre-specified cut-off *SDC2* Ct value of 39: A sample was considered positive for *SDC2* methylation if its Ct value was ⩽39 and negative for *SDC2* methylation if its Ct value was >39 or if it had no Ct value. Samples were deemed invalid if Ct value of ACTB was >36 or if there was no Ct value. In these cases, a new aliquot of the stool sample was used for reanalysis.

## FIT and detection of plasma CEA

A colloidal gold immunochromatographic assay was used for FIT and performed according to the manufacturer's instructions (W.H.P.M. Inc., Beijing, China). The result was positive if lines T and C agglutinated to each form a color band, and the result was negative if only line C formed a color band. Electrochemiluminescence immunoassay was used to detect plasma CEA on MODULAR Analysis YTICS E170, Cobas E601, and Cobas E602 (Roche). The result with plasma CEA ⩽6.5 ng/ml was called negative and otherwise positive. All tests were performed in the clinical laboratory of Songjiang Hospital.

## Statistical analysis

Wilcoxon rank sum tests were performed to compare methylation levels between the different experimental groups. Data distribution was plotted for *SDC2* after log transformation to reduce skewness. The Fisher exact test was used to evaluate the correlation between methylation levels and demographic and clinical characteristics. *P*-values <0.05 were considered statistically significant. A receiver operator characteristic (ROC) curve was constructed to compare *SDC2* methylation levels among the different groups. The associated area under the curve (AUC) value was calculated for each ROC curve. Statistical analyses were conducted with Graph Pad Prism version 6.0 (Graph Pad Software Inc, San Diego, CA). Spearman correlation analysis was further performed with SPSS 20.0 (IBM Corporation, Armonk, NY) to evaluate possible correlation between *SDC2* methylation and clinical and pathological characteristics of CRC patients.

# RESULTS

## Demographic and clinical features of CRC, AA, and control groups

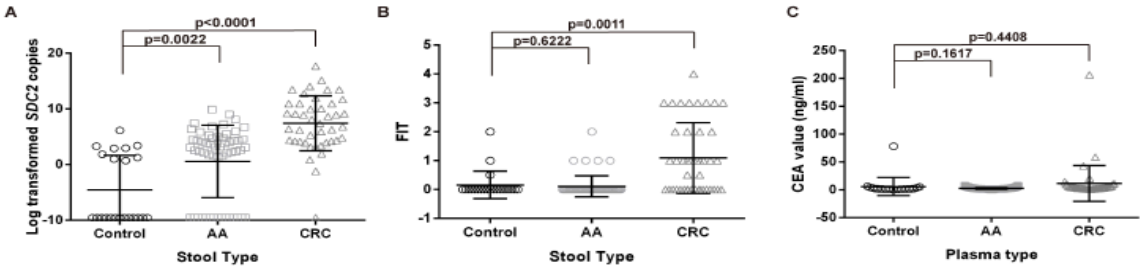


**Figure 1. Performance of the *SDC2* methylation test, FIT and plasma CEA for detection of CRC and AA VS. control.** (A) Methylation levels of *SDC2* in 43 CRC, 55 AA and 22 control samples. *SDC2* methylation level in CRC and AA groups was significantly higher than that in the control group (P< 0.001 and $P = 0.002$, respectively). (B) Performance of FIT in 43 CRC, 55 AA and 22 control stool samples. More subjects in the CRC group were FIT positive than those in the control group ($P = 0.001$), while no differences were observed between the AA and control group ($P = 0.622$). (C) Plasma CEA values in 43 CRC, 55 AA and 22 control plasma samples. No differences were observed in plasma CEA values between the CRC, or AA group and the control group. ($P = 0.441$ and $P = 0.162$, respectively).

Of the 120 study participants in the final analysis, 43 had CRC (35.8%), 55 had AA (45.8%), and 22 had neither (control; 18.3%) based on colonoscopy examinations and/or pathological reports. The control group consisted of individuals with ulcerative colitis, benign polyps, non-AA (<1cm), and negative colonoscopy findings (**Table S1-3**). The baseline demographic and clinical information for the study participants is summarized in **Table 1**. There were significant age differences between the three groups: The CRC group had the oldest median age (68) while the control group had the youngest (61.5). Significantly more men than women were present in both CRC and AA groups (25 VS 18; 38VS. 17), and more than half of the participants in the CRC group (23/43, 53.5%) had early-stage cancers (stage I/II). A large proportion of the CRC tumors and AAs were located at distal region of the colon (66/98, 67.3%), and their sizes varied widely from 0.6 to 9.0 cm. The majority of cancers were in the moderately differentiated or well differentiated state (39/43, 90.7%).

## Performance characteristics of sDNA methylation test, FIT, and plasma CEA

The median logarithmic methylation levels of *SDC2* in the CRC and AA groups were significantly higher than that in the control group ($P$<0.001 and $P$=0.002)

**Table 1. Clinical characteristics of CRC, AA and control groups**

| Variables | CRC group (n=43) | AA group (n = 55) | Control group (n = 22) |
|---|---|---|---|
| Age – yr | | | |
| median (range) | 68(46-86) | 64(34-74) | 61.5(28-72) |
| Gender – no. (%) | | | |
| Male | 25 (58.1) | 38 (69.1) | 15 (68.2) |
| Female | 18 (41.9) | 17 (30.9) | 7 (31.8) |
| Stage – no. (%) | | | |
| I/II | 23 (53.5) | N/A | N/A |
| III/IV | 20 (46.5) | | |
| Location – no. (%) | | | |
| Proximal | 12 (27.9) | 20 (36.4) ¯ | N/A |
| Distal | 31 (72.1) | 35 (63.6) ¯ | |
| Tumor size (mm) | | | |
| Median (range) | 35 (8-90) | 15 (6-50) | N/A |
| Differentiation – no. (%) | | | |
| Low | 4 (9.3) | | |
| Median | 25 (58.1) | N/A | N/A |
| High | 14 (32.6) | | |

N/A: not applicable. ¯10 subjects in the AA group had multiple polyps in both proximal and distal regions.

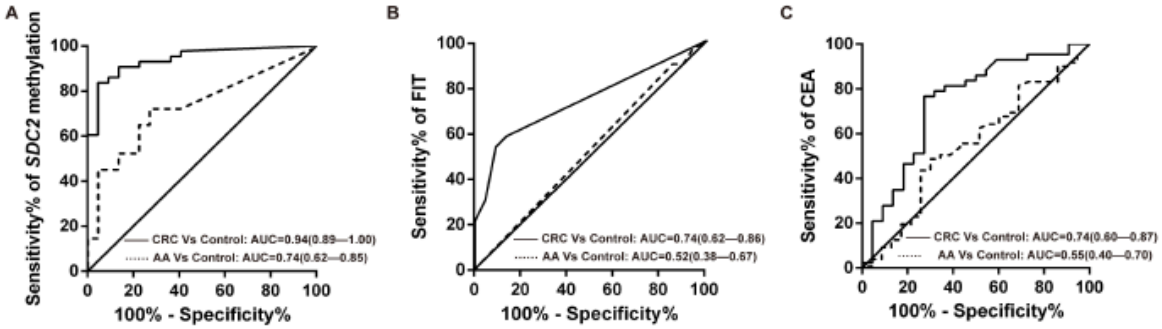


**Figure 2. Receiver operating characteristic (ROC) curves for SDC2 methylation, FIT, and plasma CEA in CRC or AA vs. control samples.** (A) ROC curves for SDC2 methylation levels in CRC or AA vs. control stool samples. The area under the curve (AUC) was 0.94 (95% CI: 0.89–1.00) in the CRC group vs. the control group and 0.74 (95% CI: 0.62–0.85) in the AA group vs. the control group. (B) ROC curves for FIT test in CRC or AA vs. control stool samples. The AUC was 0.74 (95% CI: 0.62–0.86) in the CRC group vs. the control group and 0.52 (95% CI: 0.38–0.67) in the AA group vs. the control group. (C) ROC curves for the plasma CEA test in CRC or AA vs. control plasma samples. The AUC was 0.74 (95% CI: 0.60–0.87) in the CRC group vs. the control group and 0.55 (95% CI: 0.40–0.70) in the AA group vs. the control group.

(**Figure 1A**). There was a statistically significant difference in FIT test performance for CRC vs. control (*P*=0.001) but not for AA vs. control (*P*=0.622) (**Figure 1B**). There was no difference in plasma CEA test performance between either CRC vs. control or AA vs. control (*P*=0.441 and 0.162,

**Table 2. Sensitivity and specificity of *SDC2* methylation, FIT and plasma CEA tests in three groups. (%)**

| Variables | No. | *SDC2* methylation (n=120) | | FIT (n=120) | | Plasma CEA (n=120) | | *P* value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | No. with Positive Results | Sensitivity (%) (95% CI) | No. with Positive Results | Sensitivity (%) (95% CI) | No. with Positive Results | Sensitivity (%) (95% CI) | *SDC2* vs FIT | *SDC2* vs CEA | FIT vs CEA |
| CRC | 43 | 39 | 90.7 (77.0-97.0) | 25 | 58.1 (42.2-72.9) | 9 | 20.9 (10.6-36.5) | 0.001 | 0.000 | 0.001 |
| Stage I/II | 23 | 20 | 87.0 (65.3-96.6) | 10 | 43.5 (23.9-65.1) | 1 | 4.4 (0.0-23.9) | 0.005 | 0.000 | 0.004 |
| Stage III/IV | 20 | 19 | 95.0 (73.1-99.7) | 15 | 75.0 (50.6-90.4) | 8 | 40.0 (20.0-63.6) | 0.182 | 0.000 | 0.054 |
| AA | 55 | 29 | 52.7 (38.9-66.1) | 5 | 9.1 (3.4-20.7) | 2 | 3.6 (0.0-13.6) | 0.000 | 0.000 | 0.438 |
| CRC and AA | 98 | 68 | 69.4 (59.1-78.1) | 30 | 30.6 (21.9-40.9) | 11 | 11.2 (0.1-19.6) | 0.000 | 0.000 | 0.001 |
| | | | Specificity (%) (95% CI) | | Specificity (%) (95% CI) | | Specificity (%) (95% CI) | | | |
| Controls included adenomas (<1cm), etc. | 22 | 3 | 86.4 (64.0-96.4) | 3 | 86.4 (64.0-96.4) | 2 | 90.9 (69.4-98.4) | 1.000 | 1.000 | 1.000 |

In the pairwise comparison of the three groups, the test level P value was less than 0.05/3(0.167).

respectively) (**Figure. 1C).** Additionally, the AUC of *SDC2* methylation was 0.94 (95% CI: 0.89–1.00) for the CRC vs. control groups and 0.74 (95% CI: 0.62–0.85) for the AA vs. control groups (**Figure 2A**), indicating that the *SDC2* methylation test was excellent at discriminating between CRC and control and fair at discriminating between AA and control. The AUC of FIT and plasma CEA was either equal to or below 0.74, indicating much less robust performance (**Figure 2B and C**).

A total of 71 participants with a Ct value ⩽39 were classified as having positive *SDC2* methylation in this study. Among these individuals, 39 were diagnosed with CRC, 29 were diagnosed with nonmalignant AAs, and three were false positives. Among the 39 CRC cases, there was no statistically significant difference in the ability of the sDNA test to detect early- or late-stage tumors, indicating equal detection capability for both tumor types. The sensitivity of *SDC2* methylation was 90.7% for CRC and 52.7% for AA, significantly higher than that of the FIT (58.1% and 9.1%, respectively) and plasma CEA (20.9% and 3.6%, respectively) methods (Table 2). Notably, the sDNA test also delivered a strong performance of 87.0% sensitivity in detecting early-stage CRC (stage I/II). More importantly, the sDNA test was able to uncover five cases of stage 0 CRC and eight cases of high-grade intraepithelial dysplasia, most of which were not detected by FIT or plasma CEA (**Table S2**). Even though the sDNA test did not have the highest specificity among the diagnostic tests investigated, its specificity was still respectable at 86.4% (19/22).

## Association of SDC2 gene methylation with clinical characteristics of CRC patients

Among the 43 CRC patients in the study, fecal *SDC2* methylation was not correlated with gender and tumor differentiation by either Fisher exact test or Spearman correlation analysis (**Table 3, Table S4**).

**Table 3. Association of logarithmic methylation levels of *SDC2* with clinical variables in cancer samples**

| | Gender | Age | Tumor location | Tumor size | Tumor stage | Tumor differentiation |
|---|---|---|---|---|---|---|
| Correlation Coefficient | -0.095 | -0.331* | 0.380* | 0.390** | 0.404** | -0.101 |
| *P* value sig.(2-tailed) | 0.545 | 0.030 | 0.012 | 0.010 | 0.007 | 0.520 |

*correlated ($P < 0.05$); **strongly associated ($P < 0.01$)

The sDNA test seemed to perform equally well between male and female participants, and there was no statistically significant difference in the test's capacity to detect tumors in a poorly, moderately, or highly differentiated state. Though the dichotomous methylation level of *SDC2* was not significantly associated with any of the clinical characteristics analyzed (**Table S4**), in Spearman correlation analysis, logarithmic methylation levels of *SDC2* were positively correlated with tumor stage, location, and size (measured by its maximum diameter) in patients with CRC (**Table 3**). Meanwhile, logarithmic methylation levels of *SDC2* were inversely associated with patient age at CRC diagnosis, indicating that *SDC2* methylation levels may be reduced in CRC patients with increased age. All of these correlations were statistically significant (**Table 3**). In addition, almost all tumors located at both proximal and distal regions of the colon were identified by the sDNA test (11/12 vs 28/31, respectively), suggesting that tumor location has no apparent effect on tumor detection rate, even though it was associated with decreased methylation levels (**Table S1**). Furthermore, the sDNA test could detect early-stage cancers (I/II) as well as late-stage ones (III/IV) at high rates (87.0% vs 95.0%), suggesting that hypermethylation of the SDC2 promoter would be an early and frequent event in the pathogenesis of CRC. Although larger tumors were associated with increased logarithmic methylation levels of SDC2, there was no statistically significant difference in the ability of the sDNA test to detecting large (>3 cm) vs. small (⩽3 cm) CRC tumors (22/23 vs. 17/20, P=0.324; **Table S4**).

# DISCUSSION

Biomarkers play critical roles in personalized medicine and are used to screen patients who may respond substantially to target therapies (Wang et al, 2020). Research on molecular markers in CRC detection has produced a series of potential new screening modalities (Issa and Noureddine, 2017). In China, the main screening methods for CRC include FIT, plasma CEA, and colonoscopy. The present study demonstrates that the sensitivity of CEA and FIT to detect CRC and AA was <60% in this hospital-based cohort. Although FIT screening is still widely used and has an effect on the short-term mortality rate of CRC, it has no effect on overall CRC mortality (Shaukat et al., 2013). Detection of plasma CEA is used to monitor CRC recurrence in some circumstances, but as a screening method for early CRC, it has severe limitations (Gao et al., 2018). Therefore, FIT and plasma CEA are not ideal approaches for mass screening of CRC. Meanwhile, though colonoscopy can reduce the incidence of CRC by 67% and the mortality of CRC by 65%, it is poorly accepted for screening in China due to the cumbersome intestinal preparation involved, as well as its invasiveness, discomfort, and complications including intestinal perforation and bleeding (Kahi et al., 2009). Therefore, only 14% of the targeted population actually participated in the Cancer Screening Program in urban China from 2012-2015 (Chen et al., 2019). Given its noninvasive and user-friendly nature, the sDNA test could serve as a new first-line screening choice, particularly for the colonoscopy-averse population. Furthermore, more robust performance of sDNA testing in detecting early-stage CRC and nonmalignant AA delivers it a clear advantage over FIT and plasma CEA methods. Elevated *SDC2* methylation levels can alert endoscopists to observe patients more carefully during subsequent colonoscopy procedure, especially when they are searching for small or flat lesions with poor intestinal preparation, which is not uncommon in China. The sDNA test has the potential to increase the compliance rate for CRC screening among the average-risk population and to enhance the effectiveness and accuracy of each colonoscopy examination.

The clinical performance of this fecal DNA test of *SDC2* methylation for CRC detection is robust and comparable to many of previously published reports (Ahlquist et al., 2012; Barták and Kalmár, 2017; Han et al., 2019; Itzkowitz et al., 2008; Park et al., 2018). Three false negatives from the fecal DNA test were diagnosed by colonoscopy as one rectal tumor, one cancer in the sigmoid colon, and one cancer in the ascending colon. The false negatives could be attributed to two possible factors. First, it is possible that *SDC2* was not methylated in these malignant tumors, as has been shown in some tumor tissue specimens by Feng and colleagues (Niu et al., 2017). Second, it is possible that an inadequate number of exfoliated tumor cells were collected from stools of these patients, and hence fewer DNA molecules were available for qMSP reactions. Thus, future studies should examine the effects of adding new markers, collecting multi-point samples, and deploying new detection methods to increase the sensitivity of the sDNA test.

The current study has several strengths. First, it compared the clinical performance of three NMPA-approved single-target detection methods of CRC and AA in China head-to-head. The study shows, for the first time, that a newly developed single-target sDNA test of methylated *SDC2* outperforms the sensitivity of its two widely-used competitors, FIT and plasma CEA, by an absolute margin of more than 30% and 40% for CRC and AA, respectively. Second, the clinical study was enriched with 55 AA cases, more than 50% of which showed positive *SDC2* promoter hypermethylation in the fecal test. Additionally, most stage 0 CRC cases with high grade intraepithelial dysplasia were detected by this sensitive method. This desirable outcome is particularly relevant since it demonstrates the potential of the test to screen precancerous lesions in addition to malignant tumors. Third, the current study performed a Spearman correlation analysis between logarithmic methylation levels of SDC2 and clinical and pathological features of CRC. We detected significant associations between *SDC2* methylation level and tumor size, location, staging, and age of diagnosis, providing a deeper insight into the evolving pattern of *SDC2* methylation as tumors develop and progress. Korean researchers have previously reported that the level of *SDC2* methylation in tissue specimens increases with lesion severity (Oh et al., 2017), and, consistent with this, the current study showed elevated level of *SDC2* methylation as the tumor size increased and the stage progressed. The fact that a similar correlation exists between *SDC2* methylation levels in stool and the growth and progression of tumor tissues suggests that the sDNA test is a powerful and effective method to detect methylated *SDC2* in fecal samples as well as in tumor tissues.

In summary, the sDNA test delivered a more robust performance compared to FIT and plasma CEA in a clinical setting and shows promise as an emerging and attractive option for CRC and AA detection and screening. However, there are still several limitations associated with the present investigation. First, the sample size of the current study was limited. Furthermore, the sensitivity and specificity values of the sDNA test obtained from current study only make sense when compared with the other two methods. In order to obtain accurate and statistically significant performance values, further large-scale multicenter investigations in hospital-based cohort as well as an average-risk population are required. Second, our control group was composed of a mixture of healthy individuals and patients with various gastrointestinal disorders, which may have adversely affected the specificity and therefore the accuracy of the testing. Even though a pilot study showed that a plethora of diseases did not seem to affect stool-based *SDC2* methylation detection (Yu and Sung, 2019), comprehensive and systemic data are not available to assess the exact effect of interfering diseases such as

cancers of the digestive tract on the test's performance. Third, the sDNA test was designed based on the Chinese genetic background and lifestyle, which could differ significantly in other ethnic groups (Han et al., 2019; Niu et al., 2017). Therefore, the efficacy of the *SDC2* methylation test for CRC and AA in other ethnic groups should also be investigated, enabling the development of tailored tests for clinical practice around the globe.

# REFERENCES

Ahlquist DA, Zou H, Domanico M, Mahoney DW, Yab TC, Taylor WR, Butz ML, Thibodeau SN, Rabeneck L, Paszat LF, Kinzler KW, Vogelstein B, Bjerregaard NC, Laurberg S, Sørensen HT, Berger BM, Lidgard GP. (2012). Next-generation stool DNA test accurately detects colorectal cancer and large adenomas. Gastroenterology 142(2):248-256; quiz e225-246.

Barták BK, Kalmár A, Péterfia B, Patai ÁV, Galamb O, Valcz G, Spisák S, Wichmann B, Nagy ZB, Tóth K, Tulassay Z, Igaz P, Molnár B. (2017). Colorectal adenoma and cancer detection based on altered methylation pattern of SFRP1, SFRP2, SDC2, and PRIMA1 in plasma samples. Epigenetics. 12(9):751-763.

Bibbins-Domingo K, Grossman DC, Curry SJ, Davidson KW, Epling JW, Jr., García FAR, Gillman MW, Harper DM, Kemper AR, Krist AH, Kurth AE, Landefeld CS, Mangione CM, Owens DK, Phillips WR, Phipps MG, Pignone MP, Siu AL. (2016). Screening for Colorectal Cancer: US Preventive Services Task Force Recommendation Statement. JAMA 315(23):2564-2575.

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a Cancer Journal for Clinicians 68(6):394-424.

Chen H, Li N, Ren J, Feng X, Lyu Z, Wei L, Li X, Guo L, Zheng Z, Zou S, Zhang Y, Li J, Zhang K, Chen W, Dai M, He J. 2019. Participation and yield of a population-based colorectal cancer screening programme in China. Gut 68(8):1450-1457.

Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. (2016). Cancer statistics in China, 2015. CA: a cancer journal for clinicians 66(2):115-132.
Choi S, Choi Y, Jun E, Kim IS, Kim SE, Jung SA, Oh ES. 2015. Shed syndecan-2 enhances tumorigenic activities of colon cancer cells. Oncotarget 6(6):3874-3886.

Choi S, Chung H, Hong H, Kim SY, Kim SE, Seoh JY, Moon CM, Yang EG, Oh ES. (2017). Inflammatory hypoxia induces syndecan-2 expression through IL-1β-mediated FOXO3a activation in colonic epithelia. FASEB journal: official publication of the Federation of American Societies for Experimental Biology 31(4):1516-1530.

Essner JJ, Chen E, Ekker SC. (2006). Syndecan-2. The International Journal of Biochemistry & Cell Biology 38(2):152-156.

Gao Y, Wang J, Zhou Y, Sheng S, Qian SY, Huo X. (2018). Evaluation of Serum CEA, CA19-9, CA72-4, CA125 and Ferritin as Diagnostic Markers and Factors of Clinical Parameters for Colorectal Cancer. Scientific Reports 8(1):2732.

Han YD, Oh TJ, Chung TH, Jang HW, Kim YN, An S, Kim NK. (2019). Early detection of colorectal cancer based on presence of methylated syndecan-2 (SDC2) in stool DNA. Clinical Epigenetics 11(1):51.

Imperiale TF, Ransohoff DF, Itzkowitz SH. (2014). Multitarget stool DNA testing for colorectal-cancer screening. The New England journal of medicine 371(2):187-188.

Issa IA, Noureddine M. (2017). Colorectal cancer

screening: An updated review of the available options. World journal of gastroenterology 23(28):5086-5096.

Itzkowitz S, Brand R, Jandorf L, Durkee K, Millholland J, Rabeneck L, Schroy PC, 3rd, Sontag S, Johnson D, Markowitz S, Paszat L, Berger BM. (2008). A simplified, noninvasive stool DNA test for colorectal cancer detection. The American Journal of Gastroenterology 103(11):2862-2870.

Jang B, Jung H, Choi S, Lee YH, Lee ST, Oh ES. (2017). Syndecan-2 cytoplasmic domain up-regulates matrix metalloproteinase-7 expression via the protein kinase Cγ-mediated FAK/ERK signaling pathway in colon cancer. The Journal of Biological Chemistry 292(39):16321-16332.

Jemal A, Ward EM, Johnson CJ, Cronin KA, Ma J, Ryerson B, Mariotto A, Lake AJ, Wilson R, Sherman RL, Anderson RN, Henley SJ, Kohler BA, Penberthy L, Feuer EJ, Weir HK. 2017. Annual Report to the Nation on the Status of Cancer, 1975-2014, Featuring Survival. Journal of the National Cancer Institute 109(9).

Kahi CJ, Imperiale TF, Juliar BE, Rex DK. 2009. Effect of screening colonoscopy on colorectal cancer incidence and mortality. Clinical gastroenterology and hepatology: the official clinical practice journal of the American Gastroenterological Association 7(7):770-775; quiz 711.

Kim JH, Park SC. 2018. Syndecan-2 Methylation as a New Biomarker for Early Detection of Colorectal Neoplasm. Gut and liver 12(5):479-480.

Knudsen AB, Zauber AG, Rutter CM, Naber SK, Doria-Rose VP, Pabiniak C, Johanson C, Fischer SE, Lansdorp-Vogelaar I, Kuntz KM. 2016. Estimation of Benefits, Burden, and Harms of Colorectal Cancer Screening Strategies: Modeling Study for the US Preventive Services Task Force. JAMA 315(23):2595-2609.

Lai LT, Zhan Z, Zou GM. (2018). Non-invasive colon cancer screening test in early diagnosis of colorectal cancer. American Journal of Translational Medicine. 2(1):1-18.

Levin B, Lieberman DA, McFarland B, Smith RA, Brooks D, Andrews KS, Dash C, Giardiello FM, Glick S, Levin TR, Pickhardt P, Rex DK, Thorson A, Winawer SJ. (2008). Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. CA: a cancer journal for clinicians 58(3):130-160.

Mitchell SM, Ross JP, Drew HR, Ho T, Brown GS, Saunders NF, Duesing KR, Buckley MJ, Dunne R, Beetson I, Rand KN, McEvoy A, Thomas ML, Baker RT, Wattchow DA, Young GP, Lockett TJ, Pedersen SK, Lapointe LC, Molloy PL. (2014). A panel of genes methylated with high frequency in colorectal cancer. BMC Cancer 14:54.

Mytilinaiou M, Nikitovic D, Berdiaki A, Kostouras A, Papoutsidakis A, Tsatsakis AM, Tzanakakis GN. (2017). Emerging roles of syndecan 2 in epithelial and mesenchymal cancer progression. IUBMB Life. 69(11):824-833.

Niu F, Wen J, Fu X, Li C, Zhao R, Wu S, Yu H, Liu X, Zhao X, Liu S, Wang X, Wang J, Zou H. 2017. Stool DNA Test of Methylated Syndecan-2 for the Early Detection of Colorectal Neoplasia. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 26(9):1411-1419.

Oh T, Kim N, Moon Y, Kim MS, Hoehn BD, Park CH, Kim TS, Kim NK, Chung HC, An S. 2013. Genome-wide identification and validation of a novel methylation biomarker, SDC2, for blood-based detection of colorectal cancer. The Journal of molecular diagnostics: JMD 15(4):498-507.

Oh TJ, Oh HI, Seo YY, Jeong D, Kim C, Kang HW, Han YD, Chung HC, Kim NK, An S. 2017. Feasibility of quantifying SDC2 methylation in stool DNA for early detection of colorectal cancer. Clinical Epigenetics. 9:126.

Okugawa Y, Grady WM, Goel A. 2015. Epigenetic Alterations in Colorectal Cancer: Emerging Biomarkers. Gastroenterology 149(5):1204-1225.e1212.

Ørntoft MB, Nielsen HJ, Ørntoft TF, Andersen CL. 2015. Performance of the colorectal cancer screening marker Sept9 is influenced by age, diabetes and arthritis: a nested case-control study. BMC cancer 15:819.

Park YS, Kim DS, Cho SW, Park JW, Jeon SJ, Moon TJ, Kim SH, Son BK, Oh TJ, An S, Kim JH, Chae JD. (2018). Analysis of Syndecan-2 Methylation in Bowel Lavage Fluid for the Detection of Colorectal Neoplasm. Gut and Liver 12(5):508-515.

Provenzale D, Gupta S, Ahnen DJ, Markowitz AJ, Chung DC, Mayer RJ, Regenbogen SE, Blanco AM, Bray T, Cooper G, Early DS, Ford JM, Giardiello FM, Grady W, Hall MJ, Halverson AL, Hamilton SR, Hampel H, Klapman JB, Larson DW, Lazenby AJ, Llor X, Lynch PM, Mikkelson J, Ness RM, Slavin TP, Sugandha S, Weiss JM, Dwyer MA, Ogba N. 2018. NCCN Guidelines Insights: Colorectal Cancer Screening, Version 1.2018. Journal of the National Comprehensive Cancer Network: JNCCN 16(8):939-949.

Rex DK, Boland CR, Dominitz JA, Giardiello FM, Johnson DA, Kaltenbach T, Levin TR, Lieberman D, Robertson DJ. (2017a). Colorectal Cancer Screening: Recommendations for Physicians and Patients From the U.S. Multi-Society Task Force on Colorectal Cancer. Gastroenterology 153(1):307-323.

Rex DK, Boland CR, Dominitz JA, Giardiello FM, Johnson DA, Kaltenbach T, Levin TR, Lieberman D, Robertson DJ. (2017b). Colorectal Cancer Screening: Recommendations for Physicians and Patients from the U.S. Multi-Society Task Force on Colorectal Cancer. The American Journal of Gastroenterology 112(7):1016-1030.

Shaukat A, Mongin SJ, Geisser MS, Lederle FA, Bond JH, Mandel JS, Church TR. 2013. Long-term mortality after screening for colorectal cancer. The New England Journal of Medicine 369(12):1106-1114.

Sun M, Gomes S, Chen P, Frankenberger CA, Sankarasharma D, Chung CH, Chada KK, Rosner MR. 2014. RKIP and HMGA2 regulate breast tumor survival and metastasis through lysyl oxidase and syndecan-2. Oncogene 33(27):3528-3537.

Wang J, Liu S, Wang H, Zheng L, Zhou C, Li G, et al. (2020). Robust performance of a novel stool DNA test of methylated SDC2 for colorectal cancer detection: a multi-center clinical study. Clin Epigenetics 12(1): 162. doi:10.1186/s13148-020-00954

Wang S, Tan W, Yue Y, Fang Y, Qian W, Guo S, Yu O, Huang BR, Li N. (2020). Dual Targeting of FOLR1 and TRPV6 for Therapy of Multiple Carcinomas. American Journal of Translational Medicine. 4(2):95-108.

Wolf AMD, Fontham ETH, Church TR, Flowers CR, Guerra CE, LaMonte SJ, Etzioni R, McKenna MT, Oeffinger KC, Shih YT, Walter LC, Andrews KS, Brawley OW, Brooks D, Fedewa SA, Manassaram-Baptiste D, Siegel RL, Wender RC, Smith RA. 2018. Colorectal cancer screening for average-risk adults: 2018 guideline update from the American Cancer Society. CA: a cancer journal for clinicians 68(4):250-281.

Yang H, Xia BQ, Jiang B, Wang G, Yang YP, Chen H, Li BS, Xu AG, Huang YB, Wang XY. (2013). Diagnostic value of stool DNA testing for multiple markers of colorectal cancer and advanced adenoma: a meta-analysis. Canadian Journal of Gastroenterology. 27(8):467-475.

Yu J, Sung JJ. (2019.) Differential colorectal cancer genomics between east and west. Journal of Gastroenterology and Hepatology 34(5):811-812.

Zhang Y, Suehiro Y, Shindo Y, Sakai K, Hazama S, Higaki S, Sakaida I, Oka M, Yamasaki T. (2015). Long-fragment DNA as a potential marker for stool-based detection of colorectal cancer. Oncology Letters 9(1):454-458.