Methylation of SFRP2 gene as a promising noninvasive biomarker using feces in colorectal cancer diagnosis: a systematic meta-analysis

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Methylation of secreted frizzled-related protein genes (SFRP) associated with the Wnt signaling pathway has previously been reported. However, the diagnostic role of SFRP methylation in colorectal cancer (CRC) remains unclear. A systematic search was performed to identify eligible articles for analysis. The pooled OR showed that SFRP1, SFRP2, SFRP4 and SFRP5 methylation was significantly higher in CRC and benign mucosal lesions than in normal colonic mucosa. When CRC was compared to benign mucosal lesions, SFRP1 and SFRP2 methylation had a significantly higher OR, but methylated SFRP4 and SFRP5 had a similar OR. Moreover, the pooled sensitivity, specificity and AUC (area under the curve) of methylated SFRP2 in feces of patients with CRC vs. healthy subjects was 0.71, 0.94 and 0.94, respectively. Therefore, methylation of SFRP1 and SFRP2 may be significantly correlated with CRC. However, in a study with small sample size, methylated SFRP4 and SFRP5 were not shown to be closely associated with CRC. Additionally, detection of SFRP2 methylation in feces presents a potential noninvasive biomarker for CRC diagnosis.

Colorectal cancer (CRC) is the third most common cancer and the fourth leading cause of mortality of all cancers worldwide1. Based on global cancer statistics, approximately 1.4 million new cases were diagnosed in clinics, and approximately 693,900 deaths occurred due to CRC in 20122. Although colonoscopy is the gold standard in the detection of CRC, it can miss adenomas and cancers3. Histopathological examination is the most accurate means of diagnosing CRC, and most postoperative patients have a five-year relative survival rate ranging from 50% to 90%4. It is necessary to provide promising noninvasive biomarkers with better sensitivity and specificity for the diagnosis of CRC in clinics.

Epigenetic silencing of tumor suppressor genes is an important mechanism in colorectal oncogenesis. Based on the advantages of DNA methylation, such as the convenience of detection, low cost, stability and non-invasive nature of methylation as a diagnostic biomarker, it could become a powerful biomarker for the early detection and diagnosis of colorectal cancer4. DNA methylation has previously been used as a biomarker in the detection of human diseases5,6. The secreted frizzled-related proteins (SFRPs) are associated with Frizzled (Fz) proteins as a family of soluble proteins. SFRPs can bind to Wnt proteins as antagonists to inhibit signal activation7. Inactivation of SFRP genes leads to deregulated activation of Wnt signaling, which is related to CRC8. At present, SFRP2 promoter methylation in feces can be a noninvasive biomarker for the diagnosis of CRC9,10. SFRP1 promoter methylation can also be used as a noninvasive tool in CRC diagnosis11,12.

Although the association of methylated SFRP1 and SFRP2 has been investigated in patients with CRC in a meta-analysis13,14, these methylation studies were conducted using a small sample size. In our study, we first determined whether SFRP1 and SFRP2 methylation was associated with CRC in feces and blood. Moreover, the relationship between the methylation of SFRP4 or SFRP5 and CRC was also first assessed. Thus, we conducted
a meta-analysis for all available studies to evaluate the relationships between SFRP gene methylation and CRC based on a large sample size. The pooled sensitivity, specificity and AUC were conducted to assess the diagnostic value of methylated SFRP2 as a potential non-invasive biomarker in feces.

**Results**

**Included study characteristics.** Initially, a total of 285 potentially relevant articles were identified from PubMed, EMBASE, Cochrane Library, Web of Science, ProQuest, CNKI and China Wan fang literature databases. The search procedure is shown in Fig. 1, and eligible datasets were included on the basis of the defined inclusion criteria. Finally, 28 eligible case-control studies were included in the current meta-analyses. These included 19 studies on SFRP2 methylation, 11 studies on SFRP1 methylation, 4 studies on SFRP4 methylation, and 4 studies on SFRP5 methylation. The information for SFRP1, SFRP2, SFRP4 and SFRP5 methylation was collected from eligible studies and was shown in Table S1.

**Associations of SFRP1, SFRP2, SFRP4 and SFRP5 methylation.** In the current study, significant heterogeneity was performed using the random effects model to make the results more reliable (I² > 50%). Forest plots of the different sample types for the association of methylated SFRP1 and SFRP2 were listed in Figs 2, 3, 4, 5, 6 and 7. Due to a very small sample size (CRC = 228), no association between methylated SFRP4 and SFRP5 and CRC was observed in our study (Figure S1).

**Association of SFRP1 methylation in tissue.** Our results showed that the overall OR of SFRP1 methylation in CRC was significantly higher than that in normal colonic mucosa and benign mucosal lesions (OR = 43.56, P < 0.001 and OR = 2.39, P = 0.014, respectively). These data included 7 studies of 403 CRC patients vs 296 normal colonic mucosa and 3 studies of 201 CRC patients vs 144 benign mucosal lesions (Figs 2 and 3). In addition, the pooled OR from 4 studies of 155 benign mucosal lesions vs 109 normal colonic mucosa showed that SFRP1 methylation in benign mucosal lesions was significantly higher than in normal colonic mucosa (OR = 24.44 and P < 0.001) (Fig. 4). The pooled OR from the comparison of normal colonic mucosa, benign mucosal lesions, and CRC showed that SFRP1 methylation was significantly associated with CRC.

**Association of SFRP2 methylation in tissue.** The pooled SFRP2 methylation analysis included 10 studies and 6 studies involved in the comparison of 936 CRC patients and 794 normal colonic mucosa and the comparison of 763 CRC patients and 487 benign mucosal lesions. Our results demonstrated that SFRP2 methylation in CRC had a higher OR than in normal colonic mucosa and benign mucosal lesions (OR = 31.38, P < 0.001 and OR = 4.83, P < 0.001, respectively) (Figs 5 and 6). Moreover, the pooled OR from 6 studies including 487 benign mucosal lesions and 614 normal colonic mucosa showed that SFRP1 methylation in benign mucosal lesions was significantly higher than in normal colonic mucosa (OR = 24.44 and P < 0.001) (Fig. 4). The pooled OR from the comparison of normal colonic mucosa, benign mucosal lesions, and CRC showed that SFRP2 methylation was significantly correlated with CRC.

**Association of SFRP4 and SFRP5 methylation in tissue.** In the comparison of 228 CRC patients and 225 normal colonic mucosa a meta-analysis involving 4 studies indicated that SFRP4 methylation was significantly higher in CRC than in normal colonic mucosa (OR = 58.72, P < 0.001). In addition, a significant relationship from 2 studies including 63 benign mucosal lesions and 73 normal colonic mucosa was observed between SFRP4 methylation and benign mucosal lesions (OR = 12.50, P = 0.005). However, SFRP4 methylation from 2
Figure 2. Forest plot for the association with methylated SFRP1 showing the pooled OR from 7 studies of tissue with 403 CRC and 296 normal colonic mucosa and 3 fecal studies with 83 CRC and 59 healthy subjects; OR = 43.56, P < 0.001 and OR = 29.99, P < 0.001, respectively. Abbreviations: CI, confidence interval; OR, odds ratio; CRC, colorectal cancer.

Figure 3. Forest plot for the association of SFRP1 methylation status showing the pooled OR from 3 studies of tissue with 201 CRC and 144 benign mucosal lesions, 2 studies of feces with 58 CRC and 58 benign mucosal lesions, and 1 study of blood with 72 CRC and 40 benign mucosal lesions; OR = 2.39, P = 0.014, OR = 2.10, P = 0.631, and OR = 11.25, P < 0.001, respectively. Abbreviations: CI, confidence interval; OR, odds ratio; CRC, colorectal cancer.
studies had a similar OR in 87 CRC patients and 63 benign mucosal lesions (P = 0.06) (Figure S1). Our result showed that SFRP4 methylation was not correlated with CRC. A meta-analysis of 4 studies with 228 CRC patients and 225 normal colonic mucosa revealed that the methylation of SFRP5 in CRC was significantly higher than in normal colonic mucosa (OR = 19.12, P < 0.001). In addition, a significant association from 2 studies including 63 benign mucosal lesions and 73 normal colonic mucosa was found between SFRP5 methylation and benign mucosal lesions (OR = 23.50 and P = 0.01). While no significant association was found in the comparison of 87 CRC patients and 63 benign mucosal lesions (P = 0.183) (Figure S1). We found that SFRP5 methylation was not associated with CRC. However, the results should be evaluated carefully, as only two studies with small sample sizes were included. Therefore, it is necessary to further validate the result of SFRP4 and SFRP5 methylation based on larger sample sizes in the future.

Subgroup analyses in tissue. Subgroup analyses of SFRP1 and SFRP2 methylation status with significant heterogeneity were conducted to find the differences in detection methods based on ethnicity (Caucasians and Asians), and methylation detection methods (MSP and non-MSP) in tissue (Table 1).

Impact of ethnicity in tissue. The study population consisted of Caucasian and Asian patients (Table 1). When CRC was compared to normal colonic mucosa, a subgroup ethnicity analysis showed that there was significant association between methylated SFRP1 and CRC in Asians and Caucasians (OR = 34.44, P < 0.001 and OR = 64.75, P = 0.004, respectively). Significant correlation was also observed between methylated SFRP2 and CRC in Asians and Caucasians (OR = 37.86, P < 0.001 and OR = 22.49, P = 0.001, respectively). Our results suggested that methylated SFRP1 or SFRP2 was associated with Asian and Caucasian populations with CRC.

Impact of method in tissue. In the current study, the non-MSP detection method primarily included QMSP (quantitative methylation-specific PCR) and COBRA (Combined Bisulfite Restriction Analysis) (Table 1). When CRC was compared to normal colonic mucosa, the OR of methylated SFRP2 for the MSP subgroup was 69.12 (95% CI: 18.78–254.42), and for the non-MSP subgroup the OR was 17.37 (95% CI: 6.14–49.12). In comparing CRC and benign lesions, the OR of methylated SFRP2 for the MSP subgroup was 3.38 (95% CI: 1.27–8.96), and the OR for the non-MSP subgroup was 7.24 (95% CI: 4.40–11.91). In comparing the benign mucosal lesions and normal colonic mucosa, the OR of methylated SFRP2 for the MSP subgroup was 25.57 (95% CI: 9.73–67.16), and the OR for the non-MSP subgroup was 4.88 (95% CI: 1.13–21.09). Our findings suggested that methylated SFRP2 was correlated with CRC in the MSP and non-MSP detection methods in tissue.

Impact of heterogeneity on results in tissue. We assessed the differences in heterogeneity for the methylated SFRP1 and SFRP2 based on subgroup analyses, as shown in Table 1. When CRC was compared to normal colonic mucosa, the subgroup analyses based on ethnicity showed that SFRP1 methylation had a significant heterogeneity in the Caucasians subgroup (I² = 84.4%) but not in the Asian subgroup (I² = 35.9%), which suggests that the heterogeneity was from the Caucasian population. Subgroup analyses based on ethnicity demonstrated
that SFRP2 methylation had strong heterogeneity in both the Caucasian and Asian subgroups ($I^2 = 62.7\%$ and $I^2 = 77.8\%$, respectively). In addition, subgroup analyses of detection methods revealed that SFRP2 methylation had strong heterogeneity in the MSP and non-MSP subgroups ($I^2 = 54.7\%$ and $I^2 = 82.8\%$, respectively). Our findings suggested that ethnicity and detection methods were not the sources of heterogeneity.

In the comparison of the CRC and benign mucosal lesions, subgroup analyses of SFRP2 methylation based on detection methods showed that the MSP subgroup had a larger heterogeneity ($I^2 = 70.9\%$) than the non-MSP subgroup ($I^2 = 30.2\%$), suggesting that MSP subgroup was a main source of heterogeneity. According to detection methods from the comparison of benign mucosal lesions and normal colonic mucosa, significant heterogeneity of SFRP2 methylation was found in the non-MSP subgroup ($I^2 = 91.4\%$), but not in the MSP subgroup ($I^2 = 0.0\%$). The results suggested that the heterogeneity was from the non-MSP subgroup.

**Pooled OR of SFRP1 methylation in feces and blood.** We first determined whether SFRP1 and SFRP2 methylation was associated with CRC in blood and feces. The pooled OR from 3 studies with 83 CRC and 59 normal healthy feces samples showed that methylated SFRP1 in CRC was significantly higher than in normal healthy feces (OR = 29.99, $P < 0.001$) (Fig. 2). The pooled OR from 2 studies involving 58 benign mucosal lesions feces samples and 34 normal healthy feces samples showed that methylated SFRP1 in benign mucosal lesions was significantly higher than in normal healthy feces (OR = 15.91, $P < 0.001$) (Fig. 4). However, SFRP1 methylation in 2 studies with 58 CRC and 58 benign mucosal lesions had a similar OR in the feces of patients with CRC and benign mucosal lesions ($P = 0.631$) (Fig. 3). Our findings suggested that SFRP1 methylation was not correlated with CRC in feces. Only 1 study of blood with 72 CRC and 40 benign mucosal lesions showed that SFRP1 methylation had a higher OR in blood samples from patients with CRC than in blood samples from patients with benign mucosal lesions (OR = 11.25, $P < 0.001$) (Fig. 3). However, the results should be interpreted with caution based on the small sample size. More studies based on feces and blood samples are very essential to further evaluate the association of SFRP1 methylation in feces and blood.

**Pooled OR of SFRP2 methylation in blood samples.** The pooled OR from 3 studies involving blood samples from 295 patients with CRC and 127 healthy subjects showed that methylated SFRP2 had a similar OR
in CRC and healthy subjects ($P = 0.055$) (Fig. 5). The pooled OR from 3 studies involving blood samples from 295 patients with CRC and 189 patients with benign mucosal lesions showed that methylated $SFRP2$ in CRC was significantly higher than in benign mucosal lesions ($OR = 13.66, P = 0.024$) (Fig. 6). In addition, $SFRP2$ methylation from 3 studies of 189 benign mucosal lesions and 127 healthy blood subjects showed a similar OR in benign mucosal lesions and healthy blood subjects ($P = 0.119$) (Fig. 7). Our results suggested that $SFRP2$ methylation was not correlated with CRC in blood samples. However, the results should be interpreted carefully because of the small sample size. More studies of blood samples are necessary to further evaluate the correlation of $SFRP2$ methylation.

Pooled OR of $SFRP2$ methylation in feces. The results from 10 studies of 551 CRC and 390 healthy subjects and 8 studies of feces samples, including 472 CRC vs 409 benign mucosal lesions, and 3 studies of blood samples, including 295 CRC vs 189 benign mucosal lesions; $OR = 4.83, P < 0.001$, $OR = 4.06, P < 0.001$, and $OR = 13.66, P = 0.024$, respectively. Abbreviations: CI, confidence interval; OR, odds ratio; CRC, colorectal cancer.

Diagnostic capacity of $SFRP2$ methylation in feces. The current study was also further performed to assess the diagnostic value of $SFRP2$ methylation status as a non-invasive biomarker in feces. In the diagnostic discrimination of CRCs and healthy subjects, the overall sensitivity, specificity and AUC of methylated $SFRP2$ were $0.71$ (95 CI%: 0.55–0.83), $0.63$ (95 CI%: 0.55–0.70) and $0.69$ (95 CI%: 0.55–0.80).
In feces samples (Fig. 9), the use of methylated SFRP2 as a biomarker could not accurately distinguish CRC and benign lesions. We also found that the mean methylation of the SFRP2 gene was 0.72 in feces from CRC patients, and 0.38 in feces from benign mucosal lesions patients.

**Figure 7.** Forest plot of the correlation of SFRP2 methylation status, showing the pooled OR from 6 studies of tissue samples involving 487 benign mucosal lesions vs 614 normal colonic mucosa, 9 studies of feces samples involving 428 benign mucosal lesions vs 334 normal healthy feces, and 3 studies of blood samples involving 189 benign mucosal lesions vs 127 normal healthy blood samples. OR = 13.17, P < 0.001, OR = 10.20, P < 0.001, and OR = 2.02, P = 0.119, respectively. Abbreviations: CI, confidence interval; OR, odds ratio; CRC, colorectal cancer.

| Gene  | Tissue/n | Pooled OR (95% CI) | Heterogeneity (I²) | P value | Compared group |
|-------|----------|--------------------|---------------------|---------|----------------|
| SFRP1 | Caucasians/3 | 64.73 (3.92–1070.36) | 84.40% | 0.004 | CRC vs Normal |
|       | Asians/4 | 34.44 (11.88–99.83) | 35.90% | <0.001 |                |
| SFRP2 | Caucasians/3 | 22.49 (3.31–152.96) | 62.70% | 0.001 | CRC vs Normal |
|       | Asians/7 | 37.86 (15.06–95.17) | 77.80% | <0.001 |                |
|       | MSP/6 | 69.12 (18.78–254.42) | 54.70% | <0.001 |                |
|       | Non-MSP/4 | 17.37 (6.14–49.12) | 82.80% | <0.001 |                |
| SFRP2 | MSP/3 | 3.38 (1.27–8.96) | 70.90% | 0.015 | CRC vs Benign |
|       | Non-MSP/3 | 7.24 (4.40–11.91) | 38.20% | <0.001 |                |
| SFRP2 | MSP/3 | 25.57 (9.73–67.16) | 0.00% | <0.001 | Benign vs Normal |
|       | Non-MSP/3 | 4.88 (1.13–21.09) | 91.40% | 0.034 |                |

**Table 1.** Subgroup analyses for methylated SFRP1 and SFRP2 in tissue. Subgroup analysis based on sample types (tissue, blood or feces), ethnicity (Caucasians and Asians), and methylation detection methods (MSP and Non-MSP) in tissue; MSP: Methylation Specific PCR; n stands for studies; CI: confidence interval; CRC: colorectal cancer; OR: odds ratio; Non-MSP mainly include QMSP (Quantitative methylation-specific PCR) and COBRA (Combined Bisulfite Restriction Analysis); Normal: normal colonic mucosa; Benign: benign mucosal lesions.
Discussion

CRC is a prevalent malignancy that develops from normal colon epithelium into either adenomas or serrated polyps, then progresses to cancer via multiple sequences of histological development\(^\text{22,38}\). Aberrantly methylated \textit{SFRP} genes have been reported in the pathogenesis of CRC\(^\text{22,26}\), but the diagnostic role of gene methylation has not been evaluated in CRC. We performed diagnostic estimations to test the value of gene methylation in CRC diagnosis using feces.

The current study revealed that methylated \textit{SFRP1} and \textit{SFRP2} were significantly associated with CRC. However, significant association was not observed between the \textit{SFRP4} or \textit{SFRP5} methylation status and CRC. The
results of methylated SFRP4 and SFRP5 should be interpreted with caution as the sample sizes were small, which suggests the need for additional studies with larger sample sizes in the future.

Subgroup analyses were performed to evaluate the impact of ethnicity and testing methods for methylated SFRP1 and SFRP2 in tissue. When CRC was compared to normal colonic mucosa, a subgroup analysis of ethnicity suggested that SFRP1 or SFRP2 methylation was correlated with Asian and Caucasian populations with CRC. Subgroup analyses based on detection methods revealed that SFRP2 methylation was associated with CRC in the MSP and non-MSP detection methods. In the current study, normal tissue samples for methylated SFRP genes included normal mucosa from healthy individuals and adjacent normal tissue samples (from CRC or adenomas). The adjacent normal samples might have an impure composition that could be slightly contaminated by tumor cells from CRC or adenomas, which could bias the results.

We evaluated the sources of heterogeneity based on subgroup analyses in tissue. When CRC was compared to normal colonic mucosa, subgroup analyses of ethnicity revealed that SFRP1 methylation exhibited heterogeneity in the Caucasian subgroup. In addition, the outcome of SFRP1 methylation based on ethnicity and detection methods could not reveal the sources of heterogeneity. When CRC was compared to benign mucosal lesions, subgroup analyses of detection methods suggested that the heterogeneity of SFRP2 methylation was derived from the MSP subgroup. In the comparison of benign mucosal lesions and normal colonic mucosa, we found that the heterogeneity of SFRP2 methylation was derived from the non-MSP subgroup.

We also analyzed the pooled OR of methylated SFRP1 and SFRP2 to determine whether these two genes were associated with CRC in feces and blood. Finally, our results suggested that methylated SFRP2 was correlated with CRC in feces. Moreover, some studies suggest that the detection of DNA methylation from feces can be used as a diagnostic tool in patients with CRC\(^{24,27}\). Therefore, we further evaluated the diagnostic effect of methylated SFRP2 in feces. Based on benign mucosal lesions as a control standard, the correlation of SFRP2 methylation was correlated with CRC in feces. Moreover, some studies suggest that the detection of DNA methylation from feces can be used as a diagnostic tool in patients with CRC\(^{24,27}\). Therefore, we further evaluated the diagnostic effect of methylated SFRP2 in feces. Based on benign mucosal lesions as a control standard, the correlation of SFRP2 methylation was correlated with CRC in feces.

Compared with the previous meta-analysis of SFRP1 methylation status\(^{44}\), the number of studies included in the present study (n = 11) was greater than in the previous study (n = 8). The previous study also only analyzed SFRP1 methylation in tissue samples, but our study included other sample types (blood or feces), thus, we determined whether SFRP1 methylation was correlated with CRC in feces. Finally, the previous study did not analyze the correlation of SFRP1 methylation in the comparison of benign mucosal lesions and normal colonic mucosa. Moreover, in comparison with the previous meta-analyses on SFRP2 methylation\(^{11}\), our meta-analyses included 19 studies, which was 5 more than the previous study. Our meta-analyses further studied the OR of SFRP2 methylation between cancer samples and benign mucosal lesions, which the previous meta-analyses did not. In addition, we determined whether SFRP2 methylation was associated with CRC in feces and blood. Because the diagnostic effect of methylated SFRP2 remains unclear in CRC, the present study integrated sensitivity, specificity, and AUC measures to evaluate the diagnostic role of methylated SFRP2.

There were some limitations in our study. First, although we tried to minimize selection bias using PubMed, EMBASE, Cochrane Library, Web of Science, ProQuest, CNKI and China Wanfang literature databases, studies with other languages and other styles such as conference abstracts could have been missed, which might introduce selection bias because the studies were restricted to articles published in English or Chinese. Second, the main ethnic populations in our study were Asians and Caucasians, and other ethnic groups, such as Africans, were absent. Third, the studies using blood samples were relatively small. Therefore, the sensitivity, specificity, and AUC of methylated SFRP genes in blood samples were not calculated. Blood samples were also very important because of their ease of collection in the clinic, and might be promising for identifying biomarkers for CRC diagnosis. Therefore, more studies will be essential to validate the diagnostic capacity of methylated SFRP genes in blood samples in the future. Finally, in our study, we found that methylated SFRP4 and SFRP5 were not closely associated with CRC, possibly due to the smaller sample size (CRC = 228), which suggests that more studies are necessary to validate our results in the future.

In conclusion, the current study provides strong evidence that methylated SFRP1 and SFRP2 may be significantly correlated with CRC, while methylated SFRP4 and SFRP5 may not be associated with CRC. Moreover, SFRP2 methylation has a potential diagnostic effect in feces and can be applied as a non-invasive biomarker in the clinical diagnosis of CRC. However, due to the very small sample size of methylated SFRP4 and SFRP5 in the current study, additional studies will be needed to validate the results based on larger sample sizes in the future.

Materials and Methods

Literature search. A systematic search was performed to identify the relevant literature from PubMed, EMBASE, Cochrane Library, Web of Science, ProQuest, CNKI and China Wanfang literature databases through June 2015, without language restrictions. We used the following keywords and search terms: (Wnt OR frizzled related protein OR SFRP OR secreted frizzled-related protein) and (colorectal cancer OR colorectal tumor OR colorectal carcinoma OR colorectal neoplasm) AND (methylation OR epigene*).

Selection criteria of studies included in the meta-analysis. The following inclusion criteria were used to identify eligible studies: (1) the study was a case-control study detailing the methylation detection methodology, including tissue, blood or fecal samples; (2) sufficient data could be assessed to determine the methylation frequency of SFRP genes; (3) CRC patients were diagnosed by histopathologic examinations; benign mucosal lesions included adenoma and hyperplastic polyp; and normal colonic mucosa consisted of normal healthy or...
adjacent normal tissue samples; (4) IBD samples including ulcerative colitis or Crohn's disease were removed from the analysis; and (5) if authors published more than one paper using the sample data, only the most recent publication with the largest sample size was selected. Literature was excluded if it did not meet the inclusion criteria. The present meta-analysis was reported based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement (Table S2).

Data extraction. The following data were extracted from the studies included in the meta-analysis: first author name, clinicopathological histology, type of samples, total number of cases, total number of benign mucosal lesions, total number of normal colonic mucosa samples, detection methods of methylation, and frequency of methylation.

Statistical analysis. Data were analyzed using STATA software (STATA version 12.0, Stata Corporation, College Station, TX, USA). The pooled odds ratio (OR) with corresponding 95% confidence interval (95% CI) was calculated to evaluate the association between SFRP methylation and disease. Heterogeneity was assessed by a chi-square test and Q statistic for case-control studies. The pooled data were calculated using the random effects model. An I² statistic with a value of over 50% represented significant heterogeneity, and subgroup analyses were used to identify potential sources of heterogeneity. Otherwise, the fixed effects model was used, indicating a lack of heterogeneity. The integrated sensitivity, specificity and AUC (the area under the curve of the summary receiver operating characteristic) were calculated to validate the diagnostic effect of SFRP2 methylation in feces. Although the pooled sensitivity and specificity are two of the most common and important indicators of diagnostic test in the current study.

References

1. Siegel, R., Naishadham, D. & Jemal, A. Cancer statistics, 2013. CA Cancer J Clin 63, 11–30 (2013).
2. Torre, L. A. et al. Global cancer statistics, 2012. CA Cancer J Clin 65, 87–108 (2015).
3. Compton, C. C. Colorectal carcinoma: diagnostic, prognostic, and molecular features. Mod Pathol 16, 376–388 (2003).
4. Mansour, H. Cell-free nucleic acids as noninvasive biomarkers for colorectal cancer detection. Front Genet 5, 182 (2014).
5. Zhang, X. et al. Combined detection of plasma GATA5 and SFRP2 methylation is a valid noninvasive biomarker for colorectal cancer and adenomas. World J Gastroenterol 21, 2629–2637 (2015).
6. Mikeska, T. & Craig, J. M. DNA methylation biomarkers: cancer and beyond. Genes (Basel) 5, 821–864 (2014).
7. Bovolenta, P., Esteve, P., Ruiz, J. M., Cisneros, E. & Lopez-Rios, J. Beyond Wnt inhibition: new functions of secreted Frizzled-related proteins in development and disease. J Cell Sci 121, 737–746 (2008).
8. Suzuki, H., Toyota, M., Nojima, M., Mori, M. & Imai, K. [SFRP, a family of new colorectal tumor suppressor candidate genes]. Nihon Rinsho 63, 707–719 (2005).
9. Lu, H. et al. DNA methylation analysis of SFRP2, GATA4/5, NDRG4 and VIM for the detection of colorectal cancer in fecal DNA. Oncol Lett 8, 1751–1756 (2014).
10. Zhang, H., Zhu, Y. Q., Wu, Y. Q., Zhang, P. & Qi, J. Detection of promoter hypermethylation of Wnt antagonsit genes in fecal samples for diagnosis of early colorectal cancer. World J Gastroenterol 20, 6329–6335 (2014).
11. Salehi, R., Mohammadi, M., Emami, M. H. & Salehi, A. R. Methylation pattern of SFRP1 promoter in stool sample is a potential marker for early detection of colorectal cancer. Adv Biomed Res 1, 87 (2012).
12. Zhang, W. et al. DNA stool test for colorectal cancer: hypermethylation of the secreted frizzled-related protein-1 gene. Dis Colon Rectum 50, 1618–1626 (2007).
13. Sui, C., Wang, G., Chen, Q. & Ma, J. Variation risks of SFRP2 hypermethylation between precancerous disease and colorectal cancer. Tumour Biol 35, 10457–10465 (2014).
14. Chen, Y. Z. et al. aberrant promoter methylation of the SFRP1 gene may contribute to colorectal carcinogenesis: a meta-analysis. Tumor Biol 35, 9201–9210 (2014).
15. Samaei, N. M., Yazdani, Y., Alizadeh-Navaei, R., Azadeh, H. & Farazmandfar, T. Promoter methylation analysis of WNT/beta-catenin pathway regulators and its association with expression of DNTM1 enzyme in colorectal cancer. J Biomed Sci 21, 73 (2014).
16. Voorham, Q. J. et al. Promoter methylation of Wnt-antagonist genes in polyloid and nonpolyloid colorectal adenomas. BMC Cancer 13, 603 (2013).
17. Takeo, M. et al. Expansion of CpG methylation in the SFRP2 promoter region during colorectal tumorigenesis. Acta Med Okayama 65, 169–177 (2011).
18. Tang, D. et al. Diagnostic and prognostic value of the methylation status of secreted frizzled-related protein 2 in colorectal cancer. Clin Invest Med 34, E88–95 (2011).
19. Chang, E. et al. Detection of colorectal neoplasm using promoter methylation of ITGA4, SFRP2, and p16 in stool samples: a preliminary report in Korean patients. Hepatogastroenterology 57, 720–727 (2010).
20. Pehlivan, S. et al. Gene methylation of SFRP2, P16, PDK1, HIC1, and MGMT and KRAS mutations in sporadic colorectal cancer. Cancer Genet Cytogenet 201, 128–132 (2010).
21. Nagasaki, T. et al. Analysis of fecal DNA methylation to detect gastrointestinal neoplasia. J Natl Cancer Inst 101, 1244–1258 (2009).
22. Dhir, M. et al. Epigenetic regulation of WNT signaling pathway genes in inflammatory bowel disease (IBD) associated neoplasia. J Gastrointest Surg 12, 1745–1753 (2008).
23. Oberwaldler, M. et al. SFRP2 methylation in fecal DNA—a marker for colorectal polyps. Int J Colorectal Dis 23, 15–19 (2008).
24. Leung, W. K. et al. Detection of hypermethylated DNA or cyclooxygenase-2 messenger RNA in fecal samples of patients with colorectal cancer or polyps. Am J Gastroenterol 102, 1070–1076 (2007).
25. Huang, Z. H., Li, L. H., Yang, F. & Wang, J. F. Detection of aberrant methylation in fecal DNA as a molecular screening tool for colorectal cancer and precancerous lesions. World J Gastroenterol 13, 950–954 (2007).
26. Qi, J., Zhu, Y. Q., Luo, J. & Tao, W. H. Hypermethylation and expression regulation of secreted frizzled-related protein genes in colorectal tumor. World J Gastroenterol 12, 7113–7117 (2006).
27. Muller, H. M. et al. Methylation changes in fecal DNA: a marker for colorectal cancer screening? Lancet 363, 1283–1285 (2004).
28. Tang, D., Wang, D. & Li, H. Combination analysis of hypermethylated SFRP1 and SFRP2 gene in fecal as a novel epigenetic biomarker panel for colorectal cancer screening. J Nanjing Med Univ (Engl) 22, 96–101 (2008).
29. Li, X. K., Wang, D. R., Tang, D. & Yu, H. F. Preliminary study on relation of hypermethylation of secreted frizzled-related protein (SFRP2) gene in peripheral blood with colorectal cancer. Chin J Curr Adv Gen Surg 15, 519–522 (2012).
30. Xu, M. H., Cai, K. Y. & Tu, Y. Value of Fecal DNA Methylation Analysis in Early Diagnosis of Colorectal Cancer. *Chin J Clin Gastroenterol* 24, 17–19 (2012).
31. Caldwell, G. M. *et al.* The Wnt antagonist sFRP1 in colorectal tumorigenesis. *Cancer Res* 64, 883–888 (2004).
32. Caldwell, G. M. *et al.* The Wnt antagonist sFRP1 is downregulated in premalignant large bowel adenomas. *Br J Cancer* 94, 922–927 (2006).
33. Rawson, J. B. *et al.* Promoter methylation of Wnt antagonists DKK1 and SFRP1 is associated with opposing tumor subtypes in two large populations of colorectal cancer patients. *Carcinogenesis* 32, 741–747 (2011).
34. Dong, L. J., Gu, X. Y. & Jang, Z. Detection and significance of serum SFRP1 promoter methylation status in patients with colorectal cancer. *Jiangsu Med J* 36, 2153–2155 (2010).
35. Shao, S. X. *et al.* Multi-gene methylation testing increased the positive methylation rate in colorectal cancer. *Chin J Gastrointest Surg* 15, 629–632 (2012).
36. Wang, W. *et al.* Effect of Wnt Antagonist SFRP1 in the Pathogenesis of Colorectal Cancer. *J China Med Univ* 41, 440–443 (2012).
37. Suzuki, H. *et al.* Epigenetic inactivation of SFRP genes allows constitutive WNT signaling in colorectal cancer. *Nat Genet* 36, 417–422 (2004).
38. Jass, J. R., Young, J. & Leggett, B. A. Evolution of colorectal cancer: change of pace and change of direction. *J Gastroenterol Hepatol* 17, 17–26 (2002).
39. Zintzaras, E. & Ioannidis, J. P. HEGESMA: genome search meta-analysis and heterogeneity testing. *Bioinformatics* 21, 3672–3673 (2005).
40. Higgins, J. P., Thompson, S. G., Deeks, J. J. & Altman, D. G. Measuring inconsistency in meta-analyses. *BMJ* 327, 557–560 (2003).
41. DerSimonian, R. Meta-analysis in the design and monitoring of clinical trials. *Stat Med* 15, 1277–1284 (1996).
42. Midgette, A. S., Stukel, T. A. & Littenberg, B. A meta-analytic method for summarizing diagnostic test performances: receiver-operating-characteristic-summary point estimates. *Med Decis Making* 13, 253–257 (1993).
43. Jones, C. M. & Athanasiou, T. Summary receiver operating characteristic curve analysis techniques in the evaluation of diagnostic tests. *Ann Thorac Surg* 79, 16–20 (2005).

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**Author Contributions**

Q.Y. and B.W. contributed to the study conception and design. Q.Y. and T.H. contributed to drafting of the article and final approval of the submitted version. T.H., G.Y. and X.Z. contributed to the analyses and interpretation of the data and completion of figures and tables. All authors read and approved the final manuscript.

**Additional Information**

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