Accuracy of early detection of colorectal tumours by stool methylation markers: A meta-analysis

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Abstract

AIM: To evaluate the accuracy of methylation of genes in stool samples for diagnosing colorectal tumours.

METHODS: Electronic databases including PubMed, Web of Science, Chinese Journals Full-Text Database and Wanfang Journals Full-Text Database were searched to find relevant original articles about methylated genes to be used in diagnosing colorectal tumours. A quality assessment of diagnostic accuracy studies tool (QADAS) was used to evaluate the quality of the included articles, and the Meta-disc 1.4 and SPSS 13.0 software programs were used for data analysis.

RESULTS: Thirty-seven articles met the inclusion criteria, and 4484 patients were included. The sensitivity and specificity for the detection of colorectal cancer (CRC) were 73% (95%CI: 71%-75%) and 92% (95%CI: 90%-93%), respectively. For adenoma, the sensitivity and specificity were 51% (95%CI: 47%-54%) and 92% (95%CI: 90%-93%), respectively. Pooled diagnostic performance of SFRP2 methylation for CRC provided the following results: the sensitivity was 79% (95%CI: 75%-82%), the specificity was 93% (95%CI: 90%-96%), the diagnostic OR was 47.57 (95%CI: 20.08-112.72), the area under the curve was 0.9565. Additionally, the results of accuracy of SFRP2 methylation for detecting colorectal adenomas were as follows: sensitivity was 43% (95%CI: 38%-49%), specificity was 94% (95%CI: 91%-97%), the diagnostic OR was 11.06 (95%CI: 5.77-21.18), and the area under the curve was 0.9563.

CONCLUSION: Stool-based DNA testing may be useful for noninvasively diagnosing colorectal tumours and SFRP2 methylation is a promising marker that has great potential in early CRC diagnosis.

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Key words: Colorectal carcinoma; Colorectal adenoma; Stool; Methylation; Meta-analysis

Core tip: The analysis of stool methylation markers as a non-invasive test is important for the early diagnosis of colorectal tumours. However, no consensus has been reached with regard to the role of stool methylation markers in colorectal tumour diagnosis. We performed a meta-analysis of 37 articles, and the pooled results showed that stool methylation markers could be used as a valuable diagnostic and predictive tool for colorectal tumours, and that SFRP2 methylation serves as a promising marker with great potential in early colorectal cancer diagnosis.
INTRODUCTION
Colorectal cancer (CRC) is the third most common malignancy and the second leading cause of cancer-related deaths in Western countries[1,2]. A 5-year survival rate for stage I CRC has reached 90%[3], but less than 10% for CRC cases who have distant metastases[4]. However, most CRC patients are diagnosed in the middle or late stages because no typical symptoms for the early stage of CRC exist[5]. Therefore, the diagnosis of CRC in early stages has great importance for reducing CRC mortality.

Early diagnosis of colorectal cancer will help to reduce mortality and the costs for surgery. Currently, the colonoscopy screening test is of high efficacy, but the acceptability of this procedure in the general public is rather low. As an available non-invasive method, faecal testing has a unique advantage when compared to other screening modalities. Although faecal occult blood testing (FOBT) has been confirmed to reduce mortality due to CRC, the test has little or no impact on the incidence of CRC because of its low-level sensitivity to adenoma[6], i.e., a sensitivity of only 10%-20%[7]. Compared to FOBT, the most important advantage of a methylation marker test in stool samples is its higher accuracy and sensitivity for the diagnosis of premalignant lesions of CRC[8].

DNA methylation often occurs in the early stages of CRC, and many studies have been performed on the diagnosis of colorectal tumours by determining the methylation of genes in stool samples. However, the results of these studies are variable although inspiring. Thus, this meta-analysis will be conducted to assess the accuracy of the detection of colorectal tumours by the methylation of genes in stool samples.

MATERIALS AND METHODS
Search strategy
A literature search was performed independently by two investigators (Zhang H and Qi J) using the following databases: Pubmed, Web of Science, Chinese Journals Full-Text Database and Wanfang Journals Full-Text Database. All references that were cited in these studies and all published reviews were also searched. All English and Chinese references for analysis were published before January 2014. The following keywords were used in the search strategy: “colon/rectal/colorectal”, “cancer/tumours”, “stool”, and “methylation”. In this meta-analysis, 2 × 2 tables were constructed from each study for the true-positive, false-negative, and true-negative and false-positive values.

Inclusion and exclusion criteria
Eligible studies were required to meet all of the following criteria: (1) the data were independent; (2) the CRC was diagnosed using DNA methylation analysis in stool sample; (3) the patients were diagnosed with colorectal cancer or colorectal adenomas by pathology; and (4) the colonoscopy result of the control individuals was normal. Exclusion criteria for this meta-analysis were as follows: (1) studies on secondary CRC or primary CRC with other organ metastases; and (2) studies on CRC patients receiving chemotherapy or curative surgery.

Data extraction and quality assessment
The following data were extracted from each study: author, year of publication, country or region, sample size, the name of genes, the detection method of methylation and the study design. The data were independently extracted by two investigators (Zhang H and Qi J), and discrepancies were solved by a third investigator (Zhu YQ) and collective discussion. Quality Assessment of Studies of Diagnostic Accuracy (QUADAS)[9] was used to assess the quality of the primary studies with diagnostic accuracy, and quality scoring was appraised based on the empirical evidence, the experts’ opinions and the formal consensus. Score of 1, 0 and -1 were given to the articles that were in compliance with the standards completely, unclear or out of standards, respectively, and the full score was 14.

Statistical analysis
All statistics were calculated and then combined using a random-effects model and 95%CI as effect measurements. The diagnostic odds ratio (DOR) reflects the relationship between the result of the diagnostic test and the disease. The summary receiver operation characteristic (SROC) curve displays the trade-off between sensitivity and specificity and represents a global summary of test performance. We used the $Q$-value, which is the intersection point of the SROC curve with a diagonal line from the left upper corner to the right lower corner of the receiver operation characteristic (ROC) space, which corresponds to the highest value of sensitivity and specificity for the test. The positive likelihood ratio (PLR) represents the value by which the odds of the disease increase when a test is positive, whereas the negative likelihood ratio (NLR) shows the value by which the odds of the disease decrease when a test is negative. Statistical heterogeneity was assessed using the $χ^2$ test, and alpha significance testing was performed at the two-tailed 0.05 level. The professional statistical software programs (Meta-DiSc 1.4 and SPSS 13.0) were used for analysis. Publication bias was assessed by Egger analysis.

RESULTS
The literature search retrieved 541 citations, 408 of which were excluded because they were duplicates. Of the 133 potentially eligible studies, 96 publications were excluded.
because they did not investigate colorectal tumour or human stool studies (n = 21), included no diagnostic value studies (n = 20), were reviews (n = 27) or had overlapping data (n = 28). Finally, 37 studies that focused on the target patient spectrum were included (Figure 1).

**Study characteristics**

Of the 37 studies, 7 were Chinese and 30 were English, and they included 4484 patients (Table 1). These studies were performed in 10 countries or regions (including China, the United States, the Netherlands, Spain, Japan, Germany, Iran, Hong Kong, Austria and South Korea). In these studies, 34 evaluated CRC, and 26 evaluated colorectal adenoma. Twenty-four studies focused on the methylation of a single gene, and the other 13 studies involved the methylation of multiple genes.

Genes evaluated in these studies were mainly involved in three types of regulation pathways: the Wnt pathway, the DNA damage repair pathway and other pathways. Five genes of the Wnt pathway were involved in 11 studies: secreted frizzled-related proteins (SFRP1, SFRP2, SFRP5), Adenomatous Polyposis Coli (APC) and WNT2. Two genes of the DNA damage repair pathway were involved in 7 of the studies: O-6-Methylguanine-DNA Methyltransferase (MGMT) and MutL Homologue 1 (MLH1). Twenty-nine studies involved 22 genes of other pathways: Vimentin, Oncostatin M Receptor-β (OSMR), Phosphatase and Actin Regulator 3 (PLAC3), Cyclin Dependent Kinase Inhibitor 2A (CDKN2A), Tissue Factor Pathway Inhibitor (TFPI), Hyperplastic Polyp/Protein (HIPP1), GAT4A, Human Lactoferrin (HLF), ATM, Ras Association Domain Family 2 (RAF2), R-AR2, Hypermethylated in Cancer 1 (HIC), Engrailed gene (EN1), N-Myr Downstream-Regulated Gene family (NDRG4), IGF4, T-cell differentiation protein (MAL), Spastic Paraplegia-20 (SPG20), Fibulin-1 (FBN1), AGTR1, SLT2, SEPT9 and Angiotensin II type 1 receptor gene (AGTR1).

Qualitative and quantitative methods were the two main types of methods used for methylation detection. The qualitative method included methylation-specific PCR (MSP) and methylation-specific melting curve analysis (MS-MCA). The quantitative method included Methyl-BEAMing; quantitative MSP (qMSP); MethyLight; combined bisulfite restriction analysis (COBRA); pyrosequencing; and quantitative, allele-specific, real-time target and signal amplification (QuARTS).

**Colorectal carcinoma meta-analysis**

The colorectal carcinoma results were pooled from 34 studies and are shown in Table 2. The meta-analysis showed that the sensitivity and specificity of the detection of colorectal carcinoma by the methylation of genes were 73% (95%CI: 71%-75%) and 92% (95%CI: 90%-93%), respectively. The positive likelihood ratio was 8.07 (95%CI: 6.26-10.41), the negative likelihood ratio was 0.31 (95%CI: 0.25-0.38), the diagnostic odds ratio was 31.49 (95%CI: 23.25-42.64), and the symmetric area under the curve was 0.9281.

Heterogeneity was significant for the sensitivity (P < 0.001), specificity (P = 0.0008), positive likelihood ratio (R = 0.0025), negative likelihood ratio (P < 0.001), and diagnostic odds ratios (P = 0.0340).

Of the involved regulation mechanisms, we found that DOR and AUC of the methylated genes belonging to the Wnt pathway were higher than those of genes of the DNA damage repair pathway and other pathways. The sensitivity, specificity, DOR and AUC of different methylated genes in the three types of pathways were calculated (Table 2), and the results indicated that the accuracy of faecal SFRP2 methylation in the diagnosis of colorectal carcinoma was higher than that of other genes, with a sensitivity of 79% (95%CI: 75%-82%) (Figure 2A), a specificity of 93% (95%CI: 90%-96%) (Figure 2B), a diagnostic OR of 47.57 (95%CI: 20.08-112.72), and an area under the curve of 0.9565 (Figure 2C).

**Colorectal adenoma meta-analysis**

Pooled colorectal adenoma analysis (Table 3), including 26 studies, provided the following results: the sensitivity and specificity of gene methylation for colorectal adenoma diagnosis were 51% (95%CI: 47%-54%) and 92% (95%CI: 90%-93%), respectively. The positive likelihood ratio was 5.52 (95%CI: 4.23-7.19), the negative likelihood ratio was 0.52 (95%CI: 0.44-0.61), and the diagnostic odds ratio and symmetric area under the curve were 12.61 (95%CI: 8.66-18.37) and 0.8830, respectively.

Heterogeneity was also clear regarding sensitivity (P < 0.001), specificity (P = 0.0233), positive likelihood ratio (P = 0.1166), negative likelihood ratio (P < 0.001), and diagnostic odds ratios (P = 0.0565).

The DOR and AUC of the methylated Wnt pathway genes were higher than those of the genes of the DNA damage repair pathway and other pathways when grouping all of the genes by pathway for analysis. In these regulation mechanisms, we also found that the Wnt pathway was higher than the DNA damage repair pathway and the other pathway group. The sensitivity, specificity, DOR and AUC of the different methylated genes in the three types of pathways were calculated (Table 3), and
| Ref.                  | Country/region | Methylation of genes                        | n   | CRC (+ -) | Adenoma (+ -) | Normal (+ -) | Blind design | Detection method | QUADAS score |
|----------------------|----------------|---------------------------------------------|-----|-----------|---------------|--------------|--------------|-----------------|--------------|
| Ahlquist et al. 2012 | Ireland        | Vimentin/NDRG4/BMP3/TFPI2                  | 98  | 26 4     | 18 4         | 5 41         | Yes          | QuaARTS         | 11            |
| Bosch et al. 2011    | The Netherlands| PHACTR3                                     | 185 | 40 25     | 6 13         | 4 97         | Unclear      | qMSP            | 10            |
| Ahlquist et al. 2011 | Ireland        | Vimentin/TFPI2                             | 639 | 214 38    | 51 43        | 29 264       | Yes          | QuaARTS         | 11            |
| Azzara et al. 2010   | Spain          | RAR2/P16/MGMT/AFPC                        | 98  | 25 13     | 20 20        | 0 20         | Yes          | MS-MCA          | 10            |
| Tang et al. 2011     | China          | SFRP2                                      | 262 | 142 27    | 29 34        | 2 28         | Yes          | MSP             | 9             |
| Baek et al. 2009     | South Korea    | Vimentin/MLH1                              | 149 | 15 31     | 21 5         | 5 32         | Yes          | MSP             | 9             |
| Mayer et al. 2009    | Spain          | EN1                                        | 149 | 18 42     | 6 46         | 0 37         |               |                 |               |
| Chung et al. 2009    | United States  | Vimentin                                   | 149 | 23 37     | 8 44         | 0 37         |               |                 |               |
| Li et al. 2009       | Japan          | SFRP2                                      | 253 | 53 18     | 31 18        | 9 104        | Unclear      | Methy-            | 5             |
| Melotte et al. 2009  | The Netherlands| NDRG4                                      | 150 | 42 33     | 33 3         | 72           | Yes          | qMSP            | 11            |
| Achen et al. 2009    | United States  | CDKN2A                                     | 133 | 60 9      | 21 13        | 2 28         | Yes          | MethyLight       | 8             |
| Hellebrekers et al. 2009 | The Netherlands | PHACTR3                                    | 150 | 44 31     | 31 9         | 66           | Yes          | qMSP            | 10            |
| Mayer et al. 2009    | Spain          | EN1                                        | 149 | 8 22      | 11 4         | 1 29         | Unclear      | MS-MCA          | 7             |
| Kim et al. 2009      | United States  | SFRP2                                      | 42  | 12 8      | 6 11         | 0 5          | Yes          | MSP             | 9             |
| Nagasaka et al. 2009 | Japan          | SFRP2                                      | 253 | 38 46     | 7 49         | 6 107        |               |                 |               |
| Glickner et al. 2009 | United States  | TFPI2                                      | 129 | 44 14     | 7 19         | 2 43         | Yes          | qMSP            | 12            |
| Wang et al. 2008     | China          | SFRP2                                      | 133 | 60 9      | 21 13        | 2 28         | Yes          | MethyLight       | 8             |
| Oberwaller et al. 2008 | Australia    | SFRP2                                      | 19  | NR 6      | 7 6          | 0 6          | Yes          | MethyLight       | 9             |
| Itzkowitz et al. 2008 | United States | Vimentin                                   | 98  | 13 11     | 11 2         | 12           | Yes          | MSP             | 13            |
| Huang et al. 2007    | China          | SFRP2/PHH1/MGMT                            | 97  | 50 2      | 15 6         | 1 23         | Yes          | MSP             | 8             |
| Itzkowitz et al. 2007 | United States | Vimentin/HLTF                              | 162 | 31 9      | NR 19        | 103          | Yes          | MSP             | 13            |
| Abbassazadeh et al. 2007 | Hong Kong | p16                                     | 49  | 5 20     | NR 0          | 6 20         | Unclear      | MSP             | 8             |
| Zhang et al. 2007    | Germany        | SFRP1                                      | 44  | 16 4      | 7 0          | 2 15         | Yes          | MSP             | 9             |
| Leung et al. 2007    | Hong Kong      | SFRP2/MLH1/HLTF/MLT/AM/TFPI2               | 75  | 16 4      | 18 7         | 3 27         | Yes          | MSP             | 13            |
| Petko et al. 2007    | United States  | MGMT/CDKN2A/MLH1                          | 48  | NR 16     | 13 7         | 12           | Yes          | MSP             | 9             |
| Lennard et al. 2005  | Germany        | HIC1                                       | 71  | 11 15     | 4 9          | 0 32         | Yes          | MSP             | 11            |
| Chen et al. 2005     | United States  | Vimentin/CDKN2A/MLH1                      | 263 | 43 51     | 6 6          | 4 111        | Yes          | MSP             | 11            |
| Müller et al. 2004   | Australia      | SFRP2/PHH1/MGMT                           | 39  | 20 3      | NR 8          | 8            | Unclear      | MethyLight       | 5             |
| Xu et al. 2012       | China          | SFRP2                                      | 90  | 20 10     | 15 15        | 1 29         | Unclear      | MSP             | 5             |
| Kang et al. 2011     | China          | MGMT/CDKN2A/MA1/CDKN2A/MLH1               | 119 | 64 5      | 17 7         | 2 24         | Unclear      | MSP             | 7             |
| Zhang et al. 2011    | China          | Vimentin/OSMR/TFPI2                       | 107 | 52 8      | 13 4         | 4 26         | Unclear      | MSP             | 9             |
Zhang H et al. Stool methylation markers in colorectal tumours

| Wnt pathway | DNA damage repair pathway | Other pathways | SE (95%CI) | SP (95%CI) | DOR (95%CI) | AUC |
|-------------|--------------------------|----------------|------------|------------|-------------|-----|
| Wnt pathway | DNA damage repair pathway | Other pathways | 73% (71%-75%) | 92% (80%-93%) | 31.49 (22.35-42.64) | 0.928 |
| Wnt pathway | -                        | Other pathways | 72% (68%-75%) | 93% (90%-96%) | 33.99 (17.99-60.50) | 0.931 |
| SFRP2       | -                        | -              | 42% (36%-47%) | 97% (94%-99%) | 12.87 (5.98-27.72) | 0.730 |
| SFRP2       | -                        | Other pathways | 57% (55%-59%) | 94% (93%-95%) | 20.17 (15.18-26.80) | 0.921 |
| MGMT        | -                        | -              | 79% (75%-82%) | 93% (90%-96%) | 47.57 (20.88-112.72) | 0.957 |
| MLH         | -                        | -              | 47% (40%-53%) | 95% (90%-98%) | 11.67 (5.10-26.67) | 0.709 |
| Vimentin    | -                        | -              | 28% (18%-39%) | 100% (95%-100%) | 23.68 (10.32-185.44) | 0.500 |
| -            | -                        | P16            | 49% (43%-54%) | 93% (90%-95%) | 13.81 (8.57-22.27) | 0.847 |
| -            | OSIR         | -              | 47% (40%-54%) | 95% (91%-98%) | 14.66 (7.06-42.47) | 0.225 |
| -            | P16          | -              | 50% (42%-58%) | 98% (92%-100%) | 24.39 (7.26-81.96) | 0.975 |
| SFRP2       | MGMT         | -              | 69% (66%-72%) | 94% (91%-96%) | 33.24 (16.76-65.93) | 0.946 |
| SFRP2       | MLH          | -              | 72% (68%-75%) | 94% (92%-96%) | 43.03 (20.15-91.87) | 0.951 |
| SFRP2       | MLH          | Vimentin       | 64% (60%-67%) | 93% (92%-95%) | 24.93 (15.34-40.50) | 0.928 |
| SFRP2       | MLH          | OSIR           | 65% (62%-69%) | 95% (93%-96%) | 33.10 (17.12-63.98) | 0.951 |
| SFRP2       | MLH          | P16            | 68% (64%-71%) | 95% (93%-97%) | 38.86 (20.11-67.54) | 0.952 |

SE: Sensitivity; SP: Specificity; DOR: Diagnostic odds ratios; AUC: The area under the curve; CI: Confidence interval; MLH: MutL Homologue; MGMT: O-6-Methylguanine-DNA Methyltransferase.

the results indicated that the values of DOR and AUC of P16 and SFRP2 were higher than those of other genes, but the accuracy of faecal SFRP2 methylation for the diagnosis of colorectal adenoma was higher than that of P16 according to sensitivity (Figure 3A-C).

**Meta-regression**

In the meta-regression analysis, the difference in relative diagnostic odds ratio values between the higher and lower quality studies was not significant. We also noted that the differences between blinded and non-blinded methods, qualitative and quantitative methods, single and multiple gene methylation did not reach statistical significance, indicating that these potential factors did not substantially affect the diagnostic accuracy, as shown in Table 4.

**Publication bias**

In our meta-analysis, publication bias was evaluated using the Egger test. The results showed no significant publication bias among the studies of SFRP2 methylation in faecal samples from CRC or adenoma patients (Figures 4A and B).

**DISCUSSION**

It is widely accepted that DNA methylation in stool may be valuable for increasing the rate of CRC detection at earlier stages. In the present study, we focused on the detection performance of gene methylation in stool samples for patients with colorectal tumours. Our analysis suggests that the specificity of SFRP2 methylation is high (93% for CRC and 94% for colorectal adenoma) for the detection of colorectal tumours; however, it has moderate (79%) and low sensitivity (43%) for diagnosing CRC and adenoma, respectively. Compared to FOBT, with a sensitivity of 14% for colorectal tumour diagnosis, the detection accuracy of faecal methylation biomarkers was higher as a CRC-screening method.

The diagnostic odds ratio (DOR) is an indicator of test accuracy. The value of the DOR ranges from 0 to
infinity, and higher values indicate better discriminatory test performance. In this meta-analysis, we found that the DOR of faecal SFRP2 methylation for colorectal carcinoma and adenoma were 47.57 and 11.06, respectively, which indicated a high level of overall accuracy for CRC and a low level for adenoma. The SROC curve represents an overall measure of the discriminatory power of a test. The area under the curve of 1 for any test indicates that the test is excellent. Our data showed that the area under the curve (AUC) values of the SROC curve for faecal
Table 3  Methylation of pooled genes for the diagnosis of colorectal adenomas

| Study                  | Sensitivity (95%CI) | Specificity (95%CI) | DOR (95%CI) | AUC  |
|------------------------|---------------------|---------------------|-------------|------|
| Wang 2010[14]          | 0.46 (0.33-0.59)    | 0.92 (0.90-0.93)    | 12.61 (8.66-18.37) | 0.883|
| Nagasaka 2009[22]      | 0.32 (0.20-0.46)    | 0.95 (0.92-0.97)    | 10.81 (6.43-18.16) | 0.932|
| Wang 2008[24]          | 0.29 (0.22-0.36)    | 0.93 (0.87-0.96)    | 4.42 (2.18-8.95)   | 0.614|
| Hannes 2008[25]        | 0.8 (0.4-1.0)       | 0.98 (0.92-1.00)    | 2.35 (0.14-4.83)   | -   |
| Huang 2007[27]         | 0.35 (0.25-0.45)    | 0.95 (0.91-0.98)    | 11.06 (5.77-21.18) | 0.956|
| Leung 2007[31]         | 0.12 (0.03-0.31)    | 0.90 (0.82-0.96)    | 13.07 (7.03-24.16) | 0.97 |
| Xu 2012[36]            | 0.50 (0.31-0.69)    | 0.95 (0.91-0.98)    | 5.20 (2.14-12.82)  | 0.817|
| Cheng 2007[41]         | 0.52 (0.30-0.74)    | 0.94 (0.87-0.97)    | 12.70 (6.30-25.88) | 0.947|
| Park 2011[43]          | 0.44 (0.24-0.65)    | 0.91 (0.83-0.97)    | 11.06 (5.77-21.18) | 0.941|

SE: Sensitivity; SP: Specificity; DOR: Diagnostic odds ratios; AUC: The area under the curve; MLH: MutL Homologue; MGMT: O-6-Methylguanine-DNA Methyltransferase.

Zhang H et al. Stool methylation markers in colorectal tumours
SFRP2 methylation for the diagnosis of colorectal carcinoma and adenoma were 0.9565 and 0.9563, respectively, which indicated that faecal SFRP2 methylation is an excellent diagnostic biomarker for colorectal tumours.

Because the DOR and SROC curve are not easy to use in clinical practice, the likelihood ratios are considered to be more clinically meaningful. For a high-quality diagnostic test, a PLR of > 10 or NLR < 0.1 is typically required. However, our meta-analysis showed that neither PLR nor NLR alone was adequate to confirm or exclude the diagnosis of colorectal carcinoma or adenoma. The PLR value was 9.12 in the diagnosis analysis of CRC, which suggested that patients with a positive faecal SFRP2 methylation assay had a nine-fold chance of being diagnosed with CRC rather than non-CRC. Therefore, a colonoscopy was necessary for patients with a positive faecal SFRP2 methylation assay to confirm the diagnosis of colorectal adenoma. Moreover, the NLR was 0.24 in the diagnosis analysis of CRC suggested that if a faecal SFRP2 methylation assay result was negative, the probability rate of the individual having CRC was 24%. For the diagnosis of colorectal adenoma, a PLR of 5.99 suggested a moderate necessity to consider colonoscopy for patients with a positive faecal SFRP2 methylation assay to confirm the diagnosis of colorectal adenoma. Moreover, the NLR was 0.60 in the diagnosis analysis of colorectal adenoma. These data suggest that a negative faecal SFRP2 methylation assay result should not be used alone as a justification for denying or discontinuing the screening of colorectal adenomas.

An aberrant Wnt signalling pathway is an early event in 90% of colorectal carcinomas. SFRPs are secreted glycoproteins that antagonise Wnt signalling by different direct or indirect mechanisms. Thus, the role of SFRPs as a negative regulator of Wnt signalling may have important significance in tumourigenesis. These epigenetic events are involved in the early steps of colon carcinogenesis, and changes in the status of DNA methylation are associated with early stages of the histologic progression of colon carcinoma. Our previous studies of CRC tissue showed that SFRP1 and SFRP2 were methylated in more than 80.6% of colorectal carcinomas. Therefore, faecal SFRP2 methylation could be expected to be a biomarker for the screening of colorectal tumours. Although it

Figure 3  Forest plot of SFRP2 methylation in the diagnosis of colorectal adenomas. A: Shows the sensitivity of SFRP2 methylation in stool samples for colorectal adenoma diagnosis; B: Shows the specificity of SFRP2 methylation in stool samples for colorectal adenoma diagnosis; C: Shows the summary receiver operating characteristic curves (SROC) of SFRP2 methylation assays for the diagnosis of colorectal adenomas.

Figure 4  Assessment of the publication bias in faecal SFRP2 methylation for the diagnosis of colorectal cancer (A) and adenomas (B). No significant publication biases were found in any of these studies (all P > 0.05).
**Table 4** Weighted meta-regression on the diagnostic accuracy of the methylation of genes assays

| Covariates                  | Coefficient | SE  | \( P \) value | RDOR | 95% CI       |
|-----------------------------|-------------|-----|---------------|------|--------------|
| QUADAS score\(^1\)          | 0.062       | 0.041 | 0.881         | 1.06 | (0.46-2.47)  |
| Detection method\(^2\)      | -0.146      | 0.401 | 0.719         | 0.86 | (0.38-1.96)  |
| Blinded design\(^3\)        | -0.166      | 0.364 | 0.651         | 0.85 | (0.40-1.78)  |
| Methylation genes\(^4\)     | -0.036      | 0.444 | 0.936         | 0.96 | (0.39-2.39)  |

\(^1\)QUADAS score, which was divided into studies with higher quality (QUADAS score ≥ 10) and those with lower quality (QUADAS score < 10); \(^2\)Detection method, which was divided into qualitative and quantitative assay methods; \(^3\)Blinded design: the study was included with or without blinded design; \(^4\)Methylation genes, which were divided into single gene and combination genes.

That has great potential in early CRC diagnosis.

**Background**

Colorectal cancer (CRC) is the third-most common malignancy and the second leading cause of cancer-related deaths in western countries. The diagnosis of CRC in early stages has great importance for reducing CRC mortality. Although significant advances in biomarker research have been achieved in diagnostic technologies, the current available modalities for diagnosing CRC remain suboptimal.

**Research frontiers**

DNA methylation is a highly discriminatory method to detect colorectal cancers. Our results demonstrate that SFRP2 methylation is a non-invasive modality, which shows promise for the accurate detection of CRC; however, a large number of studies are required to further confirm the role of faecal SFRP2 methylation for early and accurate CRC diagnosis.

**Comments**

Colorectal cancer (CRC) is the third most common malignancy and the second leading cause of cancer-related deaths in western countries. The diagnosis of CRC in early stages has great importance for reducing CRC mortality. Although significant advances have been achieved in diagnostic technologies, the current available modalities for diagnosing CRC remain suboptimal.

**Research frontiers**

DNA methylation often occurs during the early stages of colorectal tumours and has played an important role in oncology, especially in the early diagnosis of colorectal tumours. However, no consensus with regard to the role of stool methylation markers in colon tumour exists.

**Innovations and breakthroughs**

Stool methylation markers as an available non-invasive modality have high accuracy and sensitivity for the diagnosis of premalignant lesions of CRC. A few systematic reviews have reported that stool methylation markers in colorectal tumour diagnosis exist. This article comprehensively assesses the accuracy of methylation genes in stool samples for diagnosing colorectal tumours.

**Applications**

Analysis of DNA methylation in stool samples may be used as a non-invasive test for the diagnosis of CRC, and SFRP2 methylation is a promising marker that has great potential in early CRC diagnosis.

**Terminology**

Diagnostic odds ratio (DOR) reflects the relationship between the result of the diagnostic test and the disease. The summary receiver operation characteristic (SROC) curve displays the trade-off between sensitivity and specificity and represents a global summary of test performance. The authors used the Q-value, the intersection point of the SROC curve with a diagonal line from the lower corner to the upper corner of the receiver operation characteristic (ROC) space, which corresponds to the highest value of sensitivity and specificity for the test. The positive likelihood ratio (PLR) represents the value by which the odds of the disease increase when a test is positive, whereas negative likelihood ratio (NLR) shows the value by which the odds of the disease decrease when a test is negative.

**Peer review**

This study reviewed 37 trials to evaluate the accuracy of stool methylation genes for diagnosing colorectal tumours. Based on these analyses, the authors conclude that stool SFRP2 methylation is a promising marker that has great potential in early CRC diagnosis. The analysis was carefully performed, and the results were clearly presented and summarized and provided valuable advice for early clinical diagnosis of colorectal tumours.

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