

Association between SFRP promoter hypermethylation and different types of cancer: A systematic review and meta-analysis

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Abstract. Abnormal methylation of secreted frizzled-related proteins (SFRPs) has been observed in various human cancer types. The loss of SFRP gene expression induces the activation of the Wnt pathway and is a vital mechanism for tumorigenesis and development. The aim of the present systematic review was to assess the association between SFRP methylation and cancer risk. A meta-analysis was systematically conducted to assess the clinicopathological significance of SFRP methylation in cancer risk. The Cochrane Library, PubMed and Web of Science databases were comprehensively searched, and 83 publications with a total of 21,612 samples were selected for the meta-analysis. The pooled odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were calculated to evaluate the degree of associations between SFRP promoter methylation and cancer risk. Subgroup analysis, meta regression and sensitivity analysis were used to identify the potential sources of heterogeneity. SFRP1, SFRP2, SFRP4 and SFRP5 hypermethylation was significantly associated with cancer risk, with ORs of 8.48 (95% CI, 6.26-11.49), 8.21 (95% CI, 6.20-10.88), 11.41 (95% CI, 6.42-20.30) and 6.34 (95% CI, 3.86-10.42), respectively. SFRP2 methylation was significantly

associated with differentiation in colorectal cancer (OR, 2.16; 95% CI, 1.02-4.56). The results of the present study demonstrated that SFRP methylation may contribute to carcinogenesis, especially in certain cancer types, including hepatocellular carcinoma and colorectal cancer.

Introduction

Epigenetic regulation is described as a heritable DNA modification without changes in the DNA sequence (1). In cancer, epigenetic modifications involve DNA methylation of tumor suppressor gene (TSG) promoters, which inhibits gene transcription (2). Aberrant DNA methylation often occurs early in carcinogenesis in a number of cancer types, including breast cancer (3), colorectal cancer (4) and gastric cancer (5). Currently, the main techniques used in methylation research include third generation high-throughput sequencing, second generation high-throughput sequencing, whole genome bisulfite sequencing based on second generation sequencing, methylated DNA immunization co-precipitation sequencing, reduced representation bisulfite sequencing, gene chip detection technology and mass spectrometry detection. In addition, other techniques include methylation-specific PCR (MSP), bisulfite-treated sequencing and methylation-sensitive high-resolution melting curve analysis. These techniques offer the potential to screen highly methylated promoter genes to identify important biomarkers for tumors.

The Wnt signaling pathway is involved in cell proliferation, differentiation and fate determination (6). However, the abnormal activity of the Wnt pathway can lead to tumorigenesis (7). Previous studies have discovered that hepatocellular carcinoma (HCC) and >90% of colorectal cancer (CRC) tumors exhibit abnormal activation of the Wnt signal pathway and changes in the downstream components of the pathway (8,9).

Secreted frizzled-related proteins (SFRPs) are tumor suppressor genes involved in the Wnt signaling pathway by binding to Wnt ligands, forming a non-functional complex, and subsequently preventing the initiation of the signaling cascade (Fig. 1). To date, four mammalian SFRPs (SFRP1, 2, 4 and 5) have been identified that exhibit CpG promoter hypermethylation (10,11). The downregulation of SFRP genes by promoter methylation has been demonstrated in various

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Abbreviations: SFRP, secreted frizzled-related protein; CRC, colorectal cancer; GC, gastric cancer; HCC, hepatocellular carcinoma; BC, breast cancer; CC, cervical cancer; EC, esophageal cancer; RCC, renal cell carcinoma; MSP, methylation-specific PCR; QMSP, quantitative real-time MSP; COBRA, combined bisulfite restriction analysis; MethyLight, methylation-specific multiplex ligation-dependent probe amplification

Key words: secreted frizzled-related proteins, methylation, meta-analysis, systematic review, cancer

cancer types, including cervical cancer (12), leukemia (13) and lung cancer (14). In the study by Sui *et al* (15), a meta-analysis was performed that identified an association between SFRP2 in tissue and feces and the risk of CRC. In another meta-analysis, SFRP2 methylation was identified as a new biomarker for screening early CRC by detecting stool-based DNA methylation (16). In addition, aberrant methylation of the SFRP1 promoter has been demonstrated to contribute to colorectal carcinogenesis (17). Despite numerous investigations, the association between SFRP methylation in CRC and clinicopathological significance still needs to be clarified, and the association between SFRP promoter methylation and multiple tumors remains controversial. For example, Kloten *et al* (18) reported that SFRP1 and SFRP2 methylation had no association with breast cancer, whereas Suzuki *et al* (19) reported that SFRP1 and SFRP2 methylation was associated with breast cancer. However, the association of methylated SFRP4 and SFRP5 in tumors has not been assessed in a meta-analysis. Therefore, the aim of the present study was to conduct a meta-analysis to further analyze the association between different types of cancer and SFRP methylation.

Materials and methods

Search strategy. A literature search was performed independently by two investigators using data recorded in the PubMed, Web of Science and Cochrane Library databases prior to December 2017, and was restricted to English language publications. Combinations of the following terms were used in the search strategy: 'Frizzled-related protein' OR 'frizzled-related proteins' OR 'FRZB proteins' OR 'SFRPs' OR 'SFRP' OR 'SFRP1' OR 'SFRP2' OR 'SFRP4' OR 'SFRP5' and 'methy*' OR 'methylation' OR 'methylated' and 'neoplasm' OR 'tumor' OR 'cancer' OR 'neoplasia' OR 'carcinomas'. All eligible articles were retrieved, and their reference lists were further checked for potential additional articles.

Inclusion and exclusion criteria. Data extraction was performed independently by two reviewers. The inclusion criteria were as follows: i) The data were independent; ii) the case-control or cohort studies assessed the associations between SFRP methylation status and any type of human cancer or their clinicopathological features; iii) studies had sufficient data to calculate an odds ratio (OR) and 95% confidence interval (CI); iv) an explicit method of methylation detection was reported; v) studies were written in English; vi) only the most recent or detailed publication with a large sample size was selected to avoid duplicated publications; and vii) the number of subjects in the control groups was >5. The exclusion criteria for the meta-analysis were as follows: i) Reviews, letters, abstracts, case reports or expert opinions; ii) reports with insufficient data for calculation of OR; iii) studies regarding *in vitro* experiments or animal experiments; or iv) duplications of previous publications or replicated samples.

Data extraction and quality assessment. Two investigators extracted the following information independently from eligible studies according to the aforementioned inclusion and exclusion criteria: The first author, year of publication, region, names of genes, source of controls, detection method

of methylation, clinicopathological characteristics, sample material and number of methylated (M) and unmethylated (U) samples in cases and controls (Table SI). The meta-analysis was performed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis statement (20). The quality of studies was assessed according to the Newcastle-Ottawa Scale assessment for case-control or cohort studies (21). The scores ranged from 0 to 9 points; a score >6 points was considered to indicate a high-quality study.

Statistical analysis. The ORs and 95% CIs were calculated to examine the associations between SFRP methylation status and different types of human cancer. In all statistical tests, $P < 0.05$ was considered to indicate a statistically significant difference.

The heterogeneity across studies was assessed by the Cochran's Q test and I^2 (22). $P \leq 0.1$ or $I^2 > 50\%$ indicated significant heterogeneity across studies, and the pooled OR was calculated using the random-effects model. Otherwise, the fixed-effects model was performed. A subgroup analysis was performed to evaluate the source of the heterogeneity. Meta-regression was performed based on regions, method, cancer types, publication year (year <2010 or ≥ 2010), case sample size ($n < 50$ or $n \geq 50$) and sample materials to further explore the potential sources of heterogeneity.

Sensitivity analyses were conducted to assess the stability of the pooled OR and heterogeneity by sequentially omitting each study. Publication bias was assessed quantitatively by Egger's test and qualitatively by Begg's funnel plots (23). A combined Egger's of P -value < 0.05 with an asymmetric funnel plot suggested the presence of publication bias. Meta-analysis was conducted using Review Manager 5.2 (Cochrane Collaboration) and STATA 12.0 (StataCorp LP).

Results

Identification of relevant studies. The article selection process is presented as a flow chart in Fig. 2. Based on the search strategies, a total of 824 potentially relevant articles were identified, and 83 articles were included in the final analysis following screening. The majority of the excluded abstracts and titles were reviews or studies with insufficient data.

Study characteristics. In the present analysis, 21,612 samples from 83 articles were used to study SFRP promoter hypermethylation in 22 types of human tumors. Among the 83 articles, there were 79 case-control articles and 4 cohort articles. A total of 14 articles among the 79 case-control articles also contained cohort analyses. In addition, 46 articles focused on the methylation of a single gene, and the remaining 37 articles involved the methylation of multiple genes. SFRP1 was the focus of 61 studies (3 studies from cohort articles) (3,4,8,10-13,18,19,24-71), 56 studies focused on SFRP2 (2 studies from cohort articles) (4,5,8-10,12,13,18,19,28,29,31-33,35,37,38,42-45,53,57-59,62,65-67,71-90), 19 studies on SFRP4 (4,10,12,31,36,42-44,57,62,63,65-67,71,85,86,91) and 29 studies on SFRP5 (1 study from cohort articles) (4,8,10,12,19,24,29,31,42-45,49,53,57-59,62,63,65-67,71,78,85,86,92). In addition, of these 83 articles, 24 articles focused on CRC (4,5,9,30-35,72-85,93), 4 on leukemia (13,55-57), 4 on lung cancer (58,59,61,68),

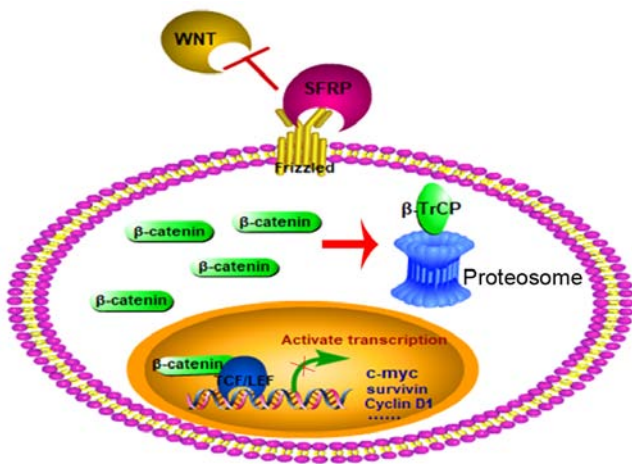


Figure 1. Pattern Diagram of SFRP blocking the WNT pathway. SFRP, secreted frizzled-related protein. β -TrCP, β -transducin repeats-containing proteins; TCF/LEF, T-cell factor/lymphocyte enhancer factor.

8 on HCC (8,49-54,88), 6 on esophageal cancer (EC) (37-42,87), 9 on gastric cancer (GC) (5,39,43-47,84,92), 5 on BC (3,18,19,28), 4 on renal cell carcinoma (RCC) (68-71) and the remaining 21 on other types of cancer. A single article evaluated SFRP methylation levels in esophageal cancer and GC (39), and 1 article evaluated SFRP methylation levels in GC and CRC (5). The information on SFRP1, SFRP2, SFRP4 and SFRP5 methylation was collected from eligible studies and presented in Table SI.

Association between SFRP1 promoter methylation and cancer risk. The results of the meta-analysis demonstrated that the frequency of SFRP1 methylation was significantly higher in patients with cancer compared with that in control samples. The pooled OR from 58 studies on SFRP1, which included 6,358 samples with various cancer types, was 8.48 (95% CI, 6.26-11.49; Table I; Fig. 3). In the analysis by cancer type, SFRP1 methylation was associated with HCC (OR, 5.00; 95% CI, 2.74-9.11; $P<0.001$), GC (OR, 10.27; 95% CI, 5.14-20.50; $P<0.001$), CRC (OR, 7.86; 95% CI, 4.87-12.68; $P<0.001$), EC (OR, 16.18; 95% CI, 3.77-69.47; $P<0.001$), RCC (OR, 12.18; 95% CI, 5.66-6.21; $P<0.001$), CC (OR, 60.61; 95% CI, 7.10-517.42; $P<0.001$), leukemia (OR, 12.85; 95% CI, 3.64-45.31; $P<0.001$), lung cancer (OR, 10.68; 95% CI, 5.94-19.20; $P<0.001$), bladder cancer (OR, 8.20; 95% CI, 3.23-20.76; $P<0.001$), ovarian cancer (OR, 22.19; 95% CI, 10.54-46.72; $P<0.001$) and endometrial carcinoma (OR, 3.07; 95% CI, 1.03-9.12; $P=0.04$), but not BC (OR, 10.70; 95% CI, 0.82-140.26; $P=0.07$). In the analysis based on method, significantly increased cancer risk was associated with SFRP1 methylation according to the MSP method (OR, 9.95; 95% CI, 7.26-13.64; $P<0.001$), COBRA method (OR, 5.48; 95% CI, 1.89-15.85; $P=0.002$), MethyLight (OR, 8.92; 95% CI, 1.10-72.12; $P=0.04$) and methylation-sensitive restriction endonuclease digestion and quantitative PCR (OR, 3.26; 95% CI, 1.33-7.95; $P=0.01$), but not by QMSP (OR, 5.01; 95% CI, 0.62-40.67; $P=0.13$). Stratified analysis by sample material revealed that significantly increased cancer risk was associated with SFRP1 methylation in tissue (OR, 9.46; 95% CI, 6.93-12.92; $P<0.001$), stool (OR, 9.33; 95% CI, 3.09-28.15; $P<0.001$) and blood (OR, 9.30; 95% CI, 3.60-24.03; $P<0.001$)

samples, but not in bone marrow samples (OR, 2.01; 95% CI, 0.35-11.69; $P=0.44$). Moreover, subgroup analysis based on region demonstrated that SFRP1 methylation was associated with cancer in patients from Asia (OR, 7.83; 95% CI, 5.70-10.76; $P<0.001$), Europe (OR, 7.58; 95% CI, 2.74-20.98; $P<0.001$) and North America (OR, 18.21; 95% CI, 9.28-35.71; $P<0.001$; Table I).

However, heterogeneity was identified across the included studies ($I^2=62\%$; $P<0.001$). Subgroup analysis was used based on sample materials, regions, cancer types and assay method to explain the sources of heterogeneity. Moderate or extensive heterogeneity still remained in the majority of the subgroups (Table I).

Association between SFRP2 promoter methylation and cancer risk. In total, 54 studies with 8,577 samples were included in the meta-analysis to assess the association between SFRP2 methylation status and cancer risk. A significant association was identified between SFRP2 promoter hypermethylation and increased cancer risk, with an OR of 8.21 (95% CI, 6.20-10.88; $P<0.001$) (Table I; Fig. 4). Analysis by cancer type revealed that significantly increased cancer risk was associated with SFRP2 methylation in HCC (OR, 1.91; 95% CI, 1.20-3.03; $P=0.006$), CRC (OR, 8.32; 95% CI, 5.88-11.78; $P<0.001$), GC (OR, 6.97; 95% CI, 2.66-18.25; $P<0.001$), EC (OR, 7.28; 95% CI, 1.72-30.79; $P=0.007$), leukemia (OR, 14.07; 95% CI, 1.84-107.61; $P=0.01$), CC (OR, 93.72; 95% CI, 29.05-302.32; $P<0.001$), ovarian cancer (OR, 19.00; 95% CI, 6.54-55.16; $P<0.001$), endometrial carcinoma (OR, 5.97; 95% CI, 2.06-17.33; $P=0.001$) and RCC (OR, 13.48; 95% CI, 5.37-33.79; $P=0.001$), but not BC (OR, 30.81; 95% CI, 0.52-1,837.06; $P=0.10$). In the subgroup analysis based on method, significantly increased cancer risk was associated with SFRP2 methylation as determined by the MSP method (OR, 8.82; 95% CI, 6.21-12.54; $P<0.001$), COBRA method (OR, 9.74; 95% CI, 5.59-16.98; $P<0.001$) and MethyLight (OR, 12.19; 95% CI, 6.92-21.48; $P<0.001$), but not according to the QMSP (OR, 5.00; 95% CI, 0.60-41.83; $P=0.14$) or reverse hybridization (OR, 2.46; 95% CI, 0.45-13.58; $P=0.30$) methods. In addition, subgroup analysis based on region reported that SFRP2 methylation was associated with cancer in patients from Asia (OR 8.69; 95% CI, 6.40-11.81; $P<0.001$), Europe (OR, 5.73; 95% CI, 1.76-18.72; $P=0.004$) and North America (OR, 6.99; 95% CI, 2.53-19.33; $P<0.001$). Stratified analysis by sample material revealed that significantly increased cancer risk was associated with SFRP2 methylation in tissue (OR, 8.29; 95% CI, 5.79-11.87; $P<0.001$), stool (OR, 9.20; 95% CI, 7.06-11.99; $P<0.001$) and blood sample (OR, 7.50; 95% CI, 2.30-24.42; $P<0.001$; Table I).

Due to significant heterogeneity among the included studies ($I^2=77\%$; $P<0.001$), subgroup analysis was performed. However, extensive heterogeneity remained in the majority of the subgroups (Table I).

Association between SFRP4 promoter methylation and cancer risks. In total, 19 studies with 2,440 samples were included in the meta-analysis to determine the effects of SFRP4 promoter hypermethylation on cancer risk. A significant association was identified between SFRP4 promoter hypermethylation and increased cancer risk with an OR of

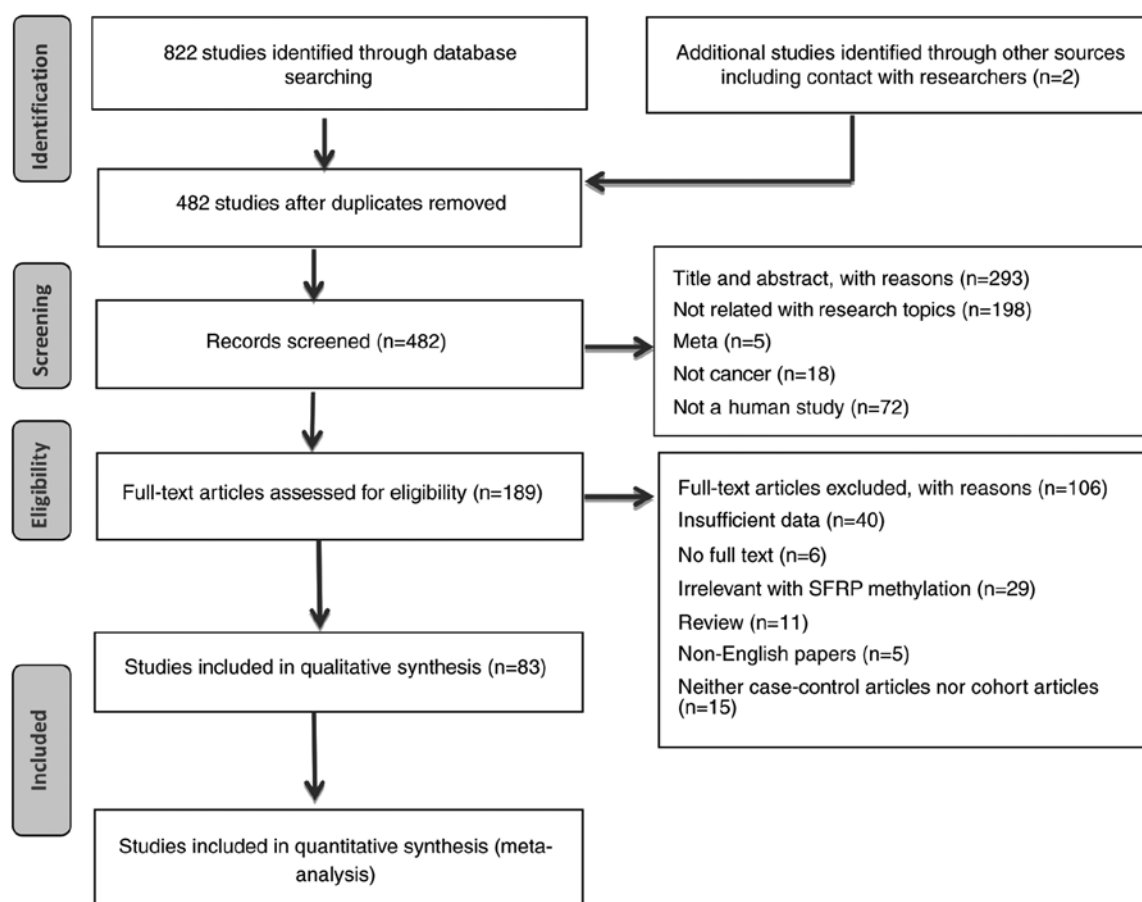


Figure 2. Flow chart of the literature search strategy. SFRP, secreted frizzled-related protein.

11.41 (95% CI, 6.42-20.30; $P < 0.001$) (Table I; Fig. 5). Analysis by cancer type revealed that significantly increased cancer risk was associated with SFRP4 methylation in CRC (OR, 11.04; 95% CI, 2.50-48.76; $P = 0.002$), ovarian cancer (OR, 22.01; 95% CI, 10.49-46.19; $P < 0.001$), CC (OR, 215.02; 95% CI, 40.42-1143.79; $P < 0.001$ and RCC (OR, 7.39; 95% CI, 3.22-16.98; $P < 0.001$), but not endometrial carcinoma (OR, 0.71; 95% CI, 0.16-3.19; $P = 0.66$) or GC (OR, 7.66; 95% CI, 0.87-67.08; $P = 0.07$). In the subgroup analysis based on method, significantly increased cancer risk was associated with SFRP1 methylation as assessed by the MSP method (OR, 11.25; 95% CI, 6.63-19.11; $P < 0.001$), but not by QMSP (OR, 25.86; 95% CI, 0.18-3638.77; $P = 0.20$). In addition, subgroup analysis based on region exhibited that SFRP4 methylation was associated with cancer in patients from Asia (OR, 11.69; 95% CI, 5.86-23.32; $P < 0.001$), and North America (OR, 9.39; 95% CI, 5.25-16.79; $P < 0.001$). Stratified analysis by sample material revealed that significantly increased cancer risk was associated with SFRP4 methylation in tissue (OR, 12.12; 95% CI, 6.50-22.59; $P < 0.001$) and blood sample (OR, 9.15; 95% CI, 1.18-70.81; $P < 0.001$; Table I).

However, evidence of heterogeneity was identified across the studies ($I^2 = 63\%$; $P < 0.001$). When subgroup analysis was performed, heterogeneity was reduced in several subgroups but remained high (Table I).

Association between SFRP5 promoter methylation and cancer risk. In total, 28 studies including 3,606 samples were

analyzed to evaluate the association of SFRP5 methylation status with various cancer types. The pooled OR of SFRP5 hypermethylation was 6.34 (95% CI, 3.86-10.42; $P < 0.001$) (Table I; Fig. 6). Analysis by cancer type revealed that significantly increased cancer risk was associated with SFRP5 methylation in HCC (OR, 4.11; 95% CI, 1.95-8.67; $P < 0.001$), CRC (OR, 6.37; 95% CI, 2.18-18.62; $P < 0.001$), ovarian cancer (OR, 59.70; 95% CI, 23.59-151.07; $P < 0.001$) and RCC (OR, 9.75; 95% CI, 4.29-22.12; $P < 0.001$), but not endometrial carcinoma (OR, 2.09; 95% CI, 0.44-10.04; $P = 0.36$), GC (OR, 3.36; 95% CI, 0.47-23.83; $P = 0.22$) and CC (OR, 11.89; 95% CI, 0.50-282.90; $P = 0.13$). In the stratified analysis based on method, significantly increased cancer risk was associated with SFRP5 methylation as determined by the MSP method (OR, 7.21; 95% CI, 4.32-12.03; $P < 0.001$). Additionally, subgroup analysis based on region demonstrated that SFRP5 methylation was associated with cancer in patients from Asia (OR, 6.54; 95% CI, 3.51-12.21; $P < 0.001$), Europe (OR, 8.69; 95% CI, 6.40-11.81; $P < 0.001$) and North America (OR, 2.08; 95% CI, 1.22-3.55; $P < 0.007$). Stratified analysis by sample material revealed that significantly increased cancer risk was associated with SFRP5 methylation in tissue (OR, 6.63; 95% CI, 3.85-11.42; $P < 0.001$) and blood sample (OR, 17.36; 95% CI, 2.29-131.85; $P < 0.006$; Table I).

High levels of heterogeneity were identified among the included studies ($I^2 = 79\%$; $P < 0.001$). When subgroup analysis was performed, moderate or extensive heterogeneity remained in most of the subgroups (Table I).

Table I. Pooled OR and 95% CI for the association between SFRP1, SFRP2, SFRP 4 and SFRP5 promoter hypermethylation and cancer risk.

Characteristic	SFRP1					SFRP2					SFRP4					SFRP5				
	n	OR (95% CI)	P-value	I ² , %	n	OR (95% CI)	P-value	I ² , %	n	OR (95% CI)	P-value	I ² , %	n	OR (95% CI)	P-value	I ² , %	n	OR (95% CI)	P-value	I ² , %
Total	58	8.48 (6.26-11.49)	<0.001	62	54	8.21 (6.20-10.88)	<0.001	77	19	11.41 (6.42-20.30)	<0.001	63	28	6.34 (3.86-10.42)	<0.001	79				
Cancer type																				
Hepatocellular carcinoma	8	5.00 (2.74-9.11)	<0.001	50	3	1.91 (1.20-3.03)	0.006	0									3	4.11 (1.95-8.67)	<0.001	0
Gastric cancer	7	10.27 (5.14-20.50)	<0.001	51	5	6.97 (2.66-18.25)	<0.001	71	2	7.66 (0.87-67.08)	0.070	89	4	3.36 (0.47-23.83)	0.220	94				
Colorectal cancer	6	7.86 (4.87-12.68)	<0.001	39	23	8.41 (5.91-11.97)	<0.001	73	3	11.04 (2.50-48.76)	0.002	67	4	6.37 (2.18-18.62)	<0.001	66				
Esophageal cancer	5	16.18 (3.77-69.47)	<0.001	80	4	7.28 (1.72-30.79)	0.007	76												
Breast cancer	4	10.70 (0.82-140.26)	0.070	88	3	30.81 (0.52-1837.06)	0.100	91												
Renal cell carcinoma	5	12.18 (5.66-26.21)	<0.001	0	2	13.48 (5.37-33.79)	<0.001	0	2	7.39 (3.22-16.98)	<0.001	0	2	9.75 (4.29-22.12)	<0.001	4				
Cervical cancer	2	60.61 (7.10-517.42)	<0.001	0	2	93.72 (29.05-302.32)	<0.001	0	3	215.02 (40.42-1143.79)	<0.001	0	2	11.89 (0.50-282.90)	0.130	87				
Ovarian cancer	2	22.19 (10.54-46.72)	<0.001	0	2	19.00 (6.54-55.16)	<0.001	77	2	22.01 (10.49-46.19)	<0.001	42	2	59.70 (23.59-151.07)	<0.001	0				
Endometrial carcinoma	2	3.07 (1.03-9.12)	0.040	0	2	5.97 (2.06-17.33)	0.001	0	2	0.71 (0.16-3.19)	0.660	47	2	2.09 (0.44-10.04)	0.360	61				
Bladder cancer	3	8.20 (3.23-20.76)	<0.001	0																
Lung cancer	3	10.68 (5.94-19.20)	<0.001	0																
Leukemia	4	12.85 (3.64-45.31)	<0.001	27	2	14.07 (1.84-107.61)	0.010	0												
Sample material																				
Tissue	42	9.46 (6.93-12.92)	<0.001	58	36	8.29 (5.79-11.87)	<0.001	79	16	12.12 (6.50-22.59)	<0.001	69	23	6.63 (3.85-11.42)	<0.001	83				
Blood	11	9.30 (3.60-24.03)	<0.001	62	7	7.50 (2.30-24.42)	<0.001	86	2	9.15 (1.18-70.81)	0.030	0	2	17.36 (2.29-131.85)	0.006	0				
Feces	2	9.33 (3.09-28.15)	<0.001	0	10	9.20 (7.06-11.99)	<0.001	42												
Bone marrow	2	2.01 (0.35-11.69)	0.440	0																
Region																				
Asia	41	7.83 (5.70-10.76)	<0.001	56	42	8.69 (6.40-11.81)	<0.001	77	13	11.69 (5.86-23.32)	<0.001	61	21	6.54 (3.51-12.21)	<0.001	81				
Europe	10	7.58 (2.74-20.98)	<0.001	77	6	5.73 (1.76-18.72)	0.004	81									2	2.08 (1.22-3.55)	0.007	28
North America	6	18.21 (9.28-35.71)	<0.001	0	5	6.99 (2.53-19.33)	<0.001	72	4	9.39 (5.25-16.79)	<0.001	0	4	11.70 (6.60-20.74)	<0.001	37				
Method																				
MSP	48	9.95 (7.26-13.64)	<0.001	55	41	8.82 (6.21-12.54)	<0.001	77	17	11.25 (6.63-19.11)	<0.001	52	25	7.21 (4.32-12.03)	<0.001	79				
COBRA	2	5.48 (1.89-15.85)	0.002	67	5	9.74 (5.59-16.98)	<0.001	74												
MethylLight	2	8.92 (1.10-72.12)	0.04	0	3	12.19 (6.92-21.48)	<0.001	0												
MSRE-qPCR	2	3.26 (1.33-7.95)	0.01	0																
QMSP	3	5.01 (0.62-40.67)	0.13	83	2	5.00 (0.60-41.83)	0.140	89	2	25.86 (0.18-3638.77)	0.20	93								
Reverse hybridization method					2	2.46 (0.45-13.58)	0.300	68												

COBRA, combined bisulfite restriction analysis; MSP, MSP, methylation-specific PCR; MSRE-qPCR, methylation-sensitive restriction endonucleases; OR, odds ratio; QMSP, real-time quantitative methylation-specific polymerase chain reaction; SFRP, secreted frizzled-related protein.

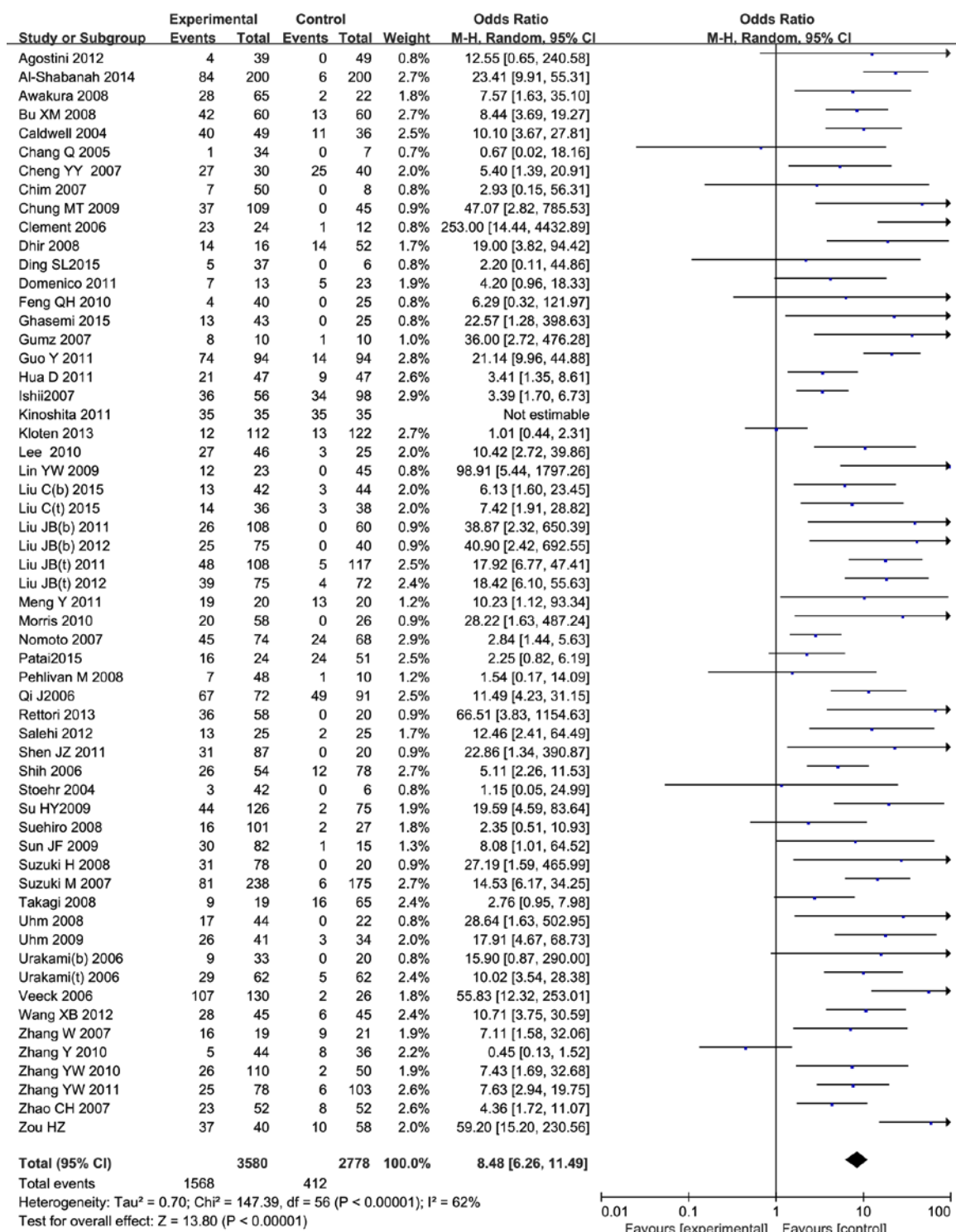


Figure 3. Forest plots of secreted frizzled-related protein 1 promoter methylation between cancer and control groups. b, blood; CI, confidence interval; M-H, Mantel-Haenszel; t, tissue.

Association between SFRP1 and SFRP2 promoter methylation and clinicopathological features of multiple cancer types. Overall, 2 studies of SFRP2 in BC, 3 studies of SFRP1 in lung cancer, 5 studies of SFRP2 in GC and 11 studies of SFRP2 in CRC provided sufficient data to assess the association between the methylation status and clinicopathological features. No associations were observed between SFRP1 methylation and certain factors, including sex, smoking habit or pathological

type in lung cancer. SFRP2 methylation was not associated with any reported clinicopathological features in BC and GC. However, SFRP2 gene promoter hypermethylation was associated with CRC tumor differentiation ('poor or other' vs. 'well or moderate': OR, 2.16; 95% CI, 1.02-4.56; P=0.04; Table SII).

Sensitivity and meta-regression analysis. Sensitivity analysis was performed by omitting each study in turn to evaluate

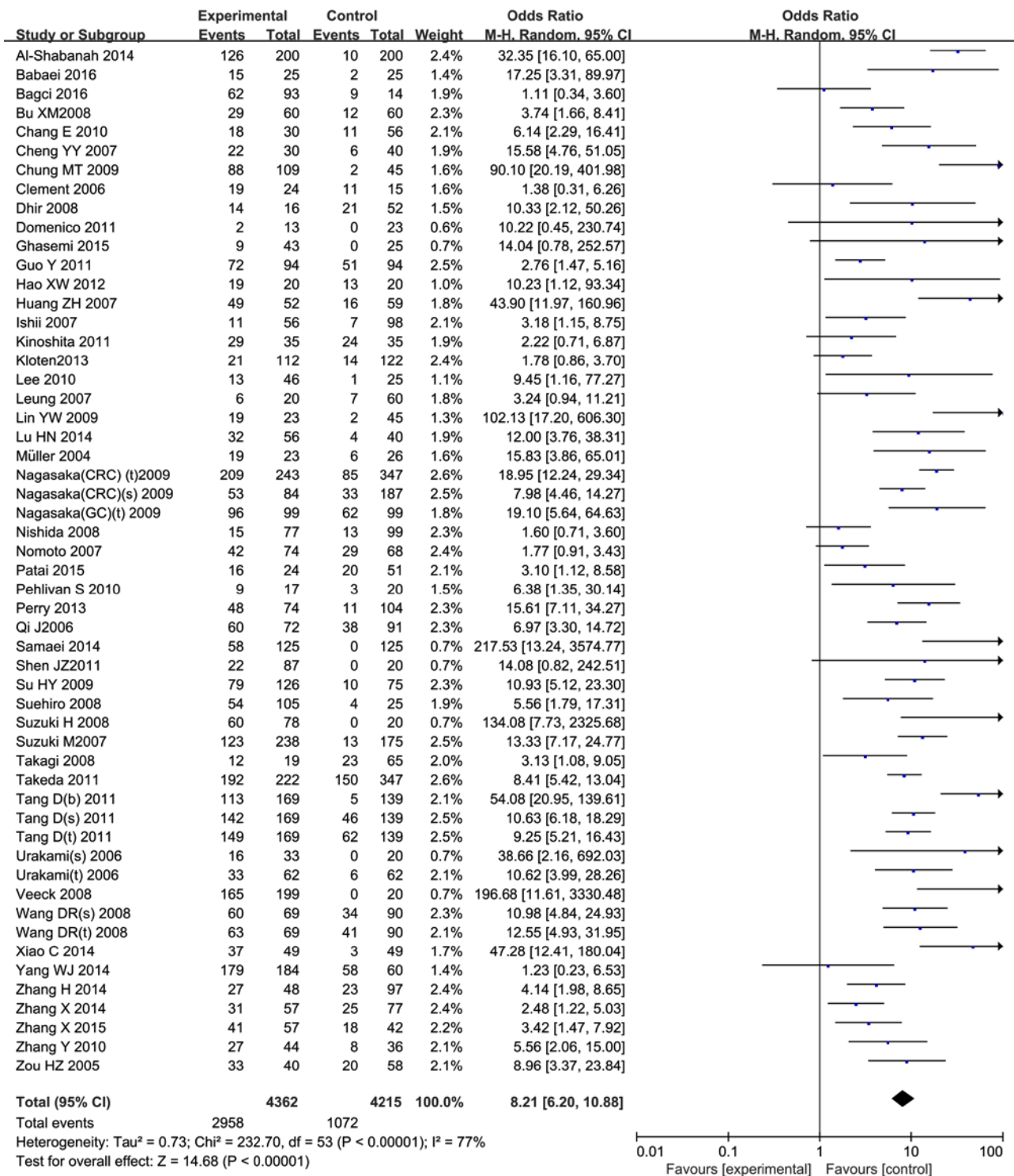


Figure 4. Forest plots of secreted frizzled-related protein 2 promoter methylation between cancer and control groups. b, blood; CI, confidence interval; CRC, colorectal cancer; GC, gastric cancer; M-H, Mantel-Haenszel; s, stool; t, tissue.

its effect on the pooled OR and to identify heterogeneous studies. To analyze the association between SFRP2 methylation and tumor differentiation in CRC, deletion of the study by Takeda *et al* (93) increased the pooled OR from 2.16 (95% CI, 1.02-4.56) to 2.70 (95% CI, 1.39-5.25), whereas the heterogeneity was reduced from 59% (P=0.02) to 39% (P=0.30).

Meta-regression analysis was conducted to explore the potential sources of heterogeneity for SFRP1, SFRP2, SFRP4

and SFRP5. The results demonstrated that sample materials may be a contributor to heterogeneity (P=0.099) for SFRP1, whereas publication year, case sample size, cancer type, region and method were not (P=0.103-0.903). In addition, publication year, sample size, region, method, cancer type and sample materials did not contribute to the heterogeneity for SFRP2 (P=0.488-0.993), SFRP4 (P=0.262-0.760) and SFRP5 (P=0.102-0.923; Table SIII).

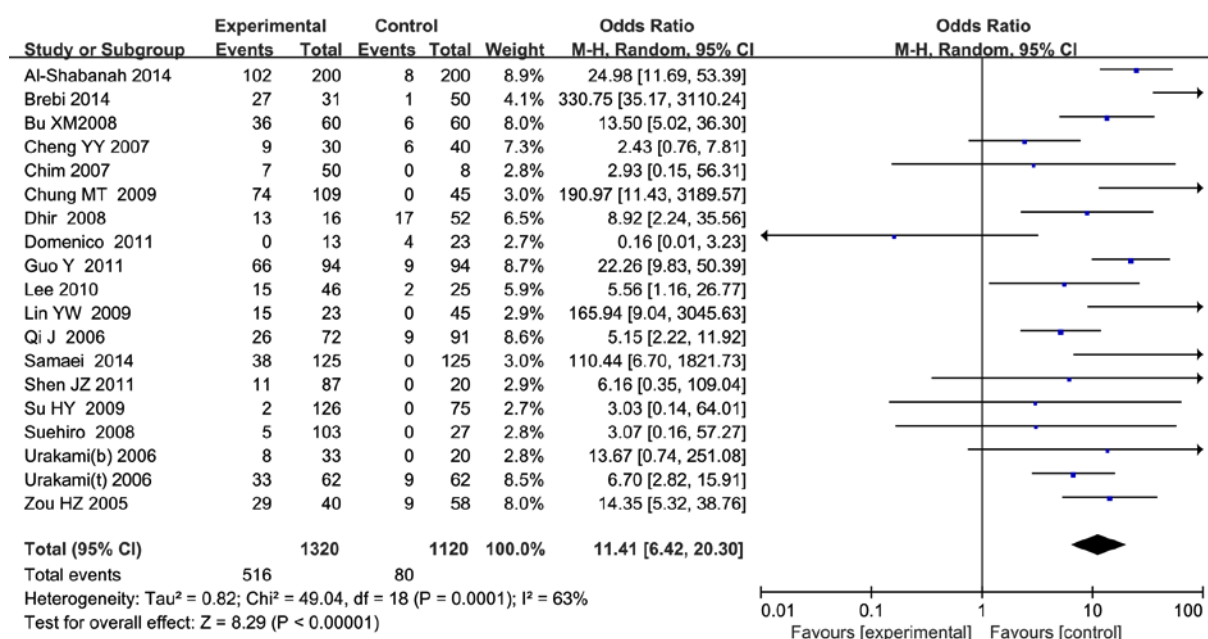


Figure 5. Forest plots of secreted frizzled-related protein 4 promoter methylation between cancer and control groups. b, blood; CI, confidence interval; M-H, Mantel-Haenszel; t, tissue.

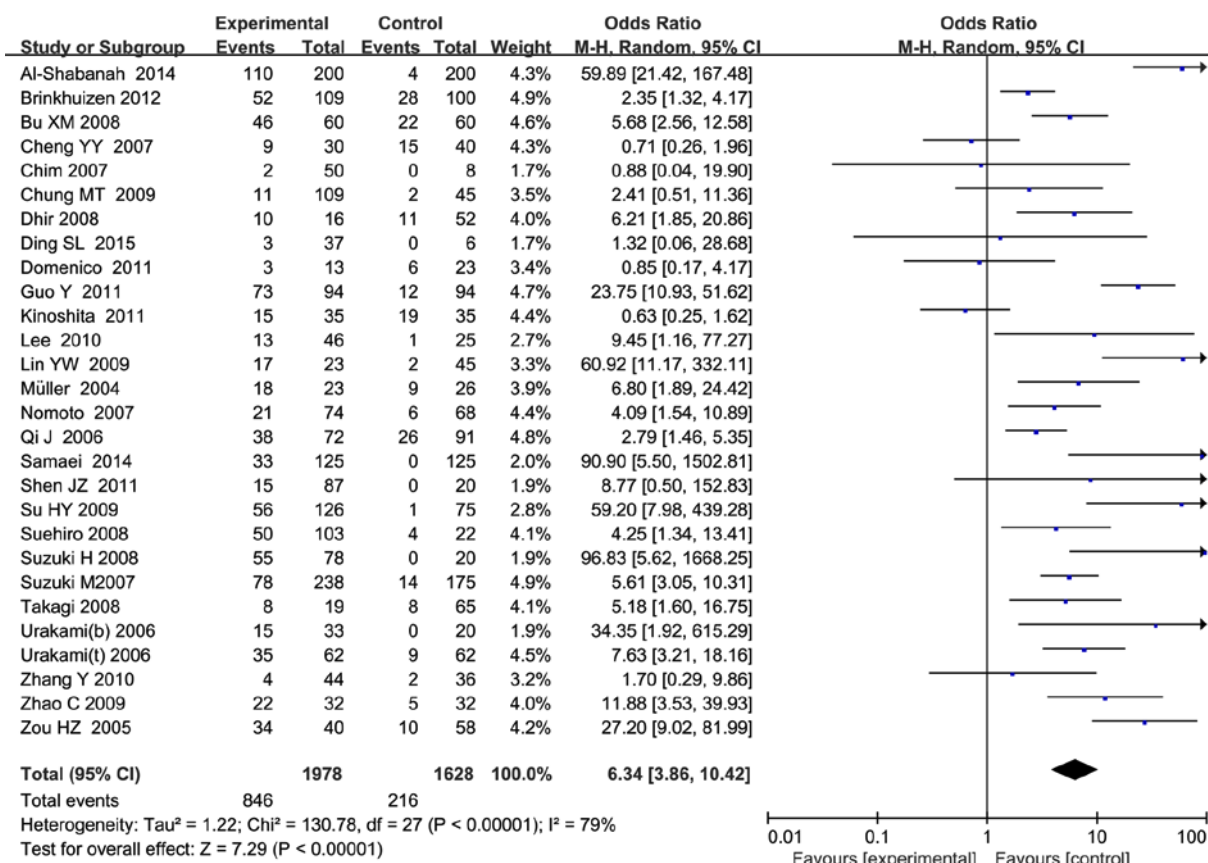


Figure 6. Forest plots of secreted frizzled-related protein 5 promoter methylation between cancer and control groups. b, blood; CI, confidence interval; M-H, Mantel-Haenszel; t, tissue.

Publication bias. Publication bias was evaluated using Begg's funnel plots and Egger's test. Begg's funnel plots did not reveal asymmetry (Fig. S1). However, publication bias was detected in the analysis of SFRP1 in various cancer types (Egger's

test, $P=0.044$; Table SIII). Therefore, a trim and fill analysis was performed to identify and amend the bias. A total of 12 adjusted studies were added to the original meta-analysis of SFRP1 with various cancer types (Fig. S1). The OR remained

significant for the association between SFRP1 methylation and risk of various cancer types (OR, 1.872; 95% CI, 1.568-2.176). Egger's tests indicated that no significant publication bias was identified for SFRP2 ($P=0.386$), SFRP4 ($P=0.992$) or SFRP5 ($P=0.254$; Table SIV).

Discussion

Hypermethylation of SFRP gene promoter is an important mechanism of Wnt signaling pathway activation, and it is also key to tumor formation and development (7). In the present study, the association between SFRP gene promoter methylation and tumor risk was analyzed. The results demonstrated that the frequency of SFRP1, SFRP2, SFRP4 and SFRP5 promoter methylation was 8.48-, 8.21-, 11.41- and 6.34-fold higher, respectively, in patients with cancer compared with that in healthy controls. Additionally, SFRP2 promoter methylation was significantly associated with poor differentiation in CRC.

A subgroup analysis based on cancer type was conducted to further investigate SFRP promoter methylation in specific tumor types. The results demonstrated that SFRP1 promoter methylation was significantly associated with HCC, GC, CRC, EC, RCC, CC, ovarian cancer, endometrial carcinoma, bladder cancer, lung cancer and leukemia. SFRP2 promoter methylation was associated with HCC, GC, CRC, EC, RCC, CC, ovarian cancer and endometrial carcinoma. Additionally, SFRP4 promoter methylation was associated with CRC, ovarian cancer, CC and RCC, and SFRP5 promoter methylation was associated with HCC, CRC, ovarian cancer and RCC. SFRP1 and SFRP2 methylation were not associated with BC. SFRP4 and SFRP5 promoter methylation was not associated with endometrial carcinoma and GC, and SFRP5 promoter methylation was not associated with CC. Therefore, despite the limited number of studies that described other specific types of cancer, SFRPs gene hypermethylation was associated with the majority of cancer types.

In the present study, SFRP methylation was significantly associated with cancer risk in tissue, blood and stool samples. The level of SFRP2 promoter methylation detected in stool samples was higher compared with that in tissue samples. A similar frequency of SFRP1 promoter methylation was detected in stool and tissue samples. The results suggested that the detection of fecal methylation biomarkers may represent a new non-invasive method to screen for malignancies. To date, studies of fecal methylation detection have mainly focused on the early diagnosis and screening of colorectal cancer (7). Stool samples contain a large number of colorectal cancer cells that escape from tumors, as well as normal colorectal epithelial cells and cell-free DNA that originates from cell degradation. The alkaline environment in the intestinal tract is conducive to DNA preservation (94). Thus, fecal DNA may be a good specimen for the molecular detection of cancer.

Subgroup analysis based on methylation detection method revealed significant associations between SFRP methylation and cancer using MSP, COBRA and MethyLight, but not QMSP. However, heterogeneity between studies remained moderately high in the MSP and COBRA subgroups. For MSP, primers based on different loci and PCR conditions used in different studies contributed to heterogeneity. COBRA can only obtain the methylation status at specific restriction

sites, and primer issues similar to those for MSP contributed to heterogeneity. Subgroup analysis based on different regions revealed that SFRP methylation was associated with cancer in patients from all covered regions. This finding indicated that although the lifestyles, environments and genetic factors were different, the relevance of SFRP methylation remained stable. However, whether geographical differences exist between specific cancer types and SFRP methylation requires further clarification.

In the subgroup analyses, the I^2 value was reduced in some stratified analyses to an extent, but it was insufficient to conclude that test method, sample material, cancer type and region may cause heterogeneity. Meta-regression was performed to explain the sources of heterogeneity; the results demonstrated that the sample material contributed to the bias among studies that investigated the association between SFRP1 methylation and multiple cancer types. For other studies, region, publication year, sample material, case sample size, cancer type and assay method did not significantly contribute to the heterogeneity.

Heterogeneity was observed in the majority of the analyses in the present study, even when the results were pooled and assessed using the random-effects model and stratification analyses based on sample material, methylation test method, cancer type and region. However, none of the previously mentioned factors contributed to the heterogeneity among the studies on SFRP2, SFRP4 and SFRP5. Publication bias was observed among the studies concerning SFRP1, but not SFRP2, SFRP4 or SFRP5 methylation. A trim and fill analysis was performed to amend the bias, and SFRP1 methylation still exhibited a significant association with cancer risk. The sensitivity analysis did not alter the significance of the association, which further supported the stability of the results.

The present study has several potential limitations. First, although the analysis was conducted with precise data extraction and strict criteria for study inclusion, heterogeneity in the subgroup analyses remained high. The possibility of unidentified confounders and selection biases could not be completely avoided. Second, the inclusion of articles only in English may have led to selection bias, as studies with potentially high-quality data published in other languages may pose difficulties in obtaining an accurate medical translation. Third, the primers based on specific locations of CpG islands and PCR conditions used to detect the status of SFRP methylation were not uniform. Fourth, some analyses were based on a limited number of studies, which led to inevitable bias. Publication bias was not identified for these studies, which subsequently influenced the gene-based analysis. However, the complete literature search identified a certain number of negative results to minimize the publication bias.

In conclusion, the results of the present meta-analysis suggested that SFRP promoter methylation may be associated with cancer risk. In addition, SFRP2 methylation was associated with CRC differentiation. Well-designed studies with larger sample sizes are needed in the future to strengthen these observations and confirm the association between SFRP promoter methylation and other cancer types.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JY and JQ conceived and designed the research. YX, ML and FW performed data acquisition, data analysis and manuscript preparation. FZ, ZZ and YL assisted with data acquisition, data analysis and statistical analysis. JY, YX and JQ contributed in writing the manuscript. JY, YX and JQ read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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