ORIGINAL RESEARCH

miR-203 as a novel biomarker for the diagnosis and prognosis of colorectal cancer: a systematic review and meta-analysis

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Abstract: We sought to systematically evaluate the diagnostic and prognostic value of miR-203 in patients with colorectal cancer. To explore the diagnostic performance of miR-203, eligible studies were identified from biomedical databases. Based on these results, 11 studies were pooled and included in this meta-analysis. The pooled sensitivity, specificity, and diagnostic odds ratios of miR-203 were 0.83 (95% confidence interval, CI: 0.78–0.86), 0.80 (95% CI: 0.77–0.83), and 19.27 (95% CI: 7.23–51.36) for the diagnosis of colorectal cancer. The area under the curve for miR-203 for diagnosing colorectal cancer was 0.89. Patients with higher expression of tissue miR-203 had poor overall survival (pooled hazard ratio: 1.63; 95% CI: 1.03–2.57, P=0.04), but serum miR-203 was not predictive (pooled hazard ratio: 1.59; 95% CI: 0.31–8.12, P=0.58). The miR-203 values of tissue and serum merged together may perhaps predict superior overall survival (pooled hazard ratio: 1.62; 95% CI: 0.93–2.82), but the effect was not significant (P=0.09).

Keywords: colorectal cancer, CRC, diagnosis, miR-203, prognosis

Introduction

Colorectal cancer (CRC) is one of the most common cancers and a leading cause of death worldwide.[1] Currently, surgery is the primary treatment, while supplementary treatments include chemotherapy and molecularly targeted therapy.[1,2] Most diagnoses of CRC occur at an advanced stage of the disease, leading to a poor prognosis and low rate of cure.[3] microRNAs (miRNAs) are 21-25nt single-stranded, non-coding RNAs, that are highly conserved in evolution. They decrease target gene expression by inhibiting mRNA translation or inducing degradation of the mRNA.[4] Calin et al[5] published the first study linking miRNAs to cancer, and these agents now have been proven to be closely related to the occurrence and development of tumors. Recent studies have reported altered expression of many miRNAs in CRC tissues, including miR-135b, miR-133, miR-21, miR-203, and miR-106a.[6–8]

Currently, the biological function of miR-203 in colorectal cancer is controversial and contradictory. Both Lin et al[9] and Yantiss et al[10] found that the miR-203 level in cancerous tissues was significantly higher than that in adjacent normal tissues, and the prognosis was poor if high levels of miR-203 were present. In contrast, Chiang et al[11] reported opposite results in 212 clinical specimens of colorectal cancers; they found that the miR-203 level in cancer tissue was lower than in the adjacent areas and 5-year survival rate of patients with lower miR-203 was worse. Thus, the mechanisms of miRNAs in cancer are not clear, and there is a need for further research on the

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role of miR-203 in colorectal cancer. The present study was designed to comprehensively explore the utility of miR-203 in colorectal cancer patients.

Materials and methods
Meta-analysis
This meta-analysis was designed, conducted, and reported according to the PRISMA statement.[12] The analytic process was carried out in accordance with the Cochrane Handbook for Systematic Reviews of Intervention.[13] The review has been registered in an international registry of systematic reviews PROSPERO (CRD42013005119).

Literature search and study selection
A comprehensive literature search was conducted in PUBMED, EMBASE, and OVID to identify eligible studies up to October 2016. The following keywords were used in the searches: ("colorectal" OR "colon" OR "rectal") AND ("cancer" OR "tumor" OR "carcinoma" OR "adenocarcinoma") AND ("microRNA" OR "microrna" OR "miR" OR "microRNA-203" OR "miR-203"). References to relevant articles and reviews were also scanned to include possible missed articles. Titles and abstracts were first scanned, and then the full papers of potential eligible studies were reviewed. Meeting abstracts were excluded because of the limited data. Articles as full papers in English were evaluated for eligibility. The retrieved studies were carefully examined to exclude potential duplicates or overlapping data.

Articles were included if they met all the following criteria: 1) the study evaluated the diagnostic or prognostic value of serum or tissue miR-203 level in CRC patients. 2) for diagnostic studies, histologic assessment was applied as reference standard for CRC; and 3) for studies that analyzed the diagnostic value of miR-203, the absolute number of true-positive (TP), false-positive (FP), true-negative (TN), and false-negative (FN) were reported or could be calculated; for prognostic studies, the hazard ratio (HR) or risk ratio values with 95% CI were provided or could be calculated.

Data extraction
Three reviewers independently collected data using standardized forms, and discrepancies were resolved by a fourth investigator. The following information from each study was extracted: first author, year of publication, origin of the study population, patient characteristics (age, sex, cancer type, and stage), source of the samples, number of participants, miR-203 assay method, follow-up time, and the variables

adjusted for in the analysis. For diagnostic studies, the numbers of TP, FP, TN, and FN results were extracted. For prognostic studies, HR estimates with 95% CI for overall survival (OS) were extracted. If the HRs and their 95% CIs were not provided, the numbers of deaths or recurrences and total samples in each study were extracted to calculate these numbers.

Quality assessment
The quality of each diagnostic study was assessed independently by three investigators according to the Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2).[14] The QUADAS-2 is recognized as an improved, redesigned tool which comprises four key domains (patient selection, index test, reference standard, and flow and timing) supported by signaling questions to aid judgment on risk of bias, rating risk of bias and concerns about applicability as "high," "unclear," and "low," and handling studies in which the reference standard consists of follow-up. For prognostic studies, the Newcastle–Ottawa scale (NOS) was applied to assess the risk of bias and the criteria for reporting observational studies to complete the methodological evaluation.[15] These scales were used to allocate a maximum of nine stars for quality of selection, comparability, exposure, and outcome of study participants. Studies with six or more stars are rated as high quality.

Statistical analysis
All statistical analyses were performed using Meta-DiSc 1.4 (XI Cochrane Colloquium, Barcelona, Spain), Review Manager 5.2 (The Nordic Cochrane Center, Rigshospitalet, Denmark), or STATA 12.0 (Stata Corporation, College Station, TX, USA) software. All accuracy data from each study (TP, FP, TN, and FN) were extracted to obtain pooled sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), positive predicted value, negative predicted value, diagnostic odds ratio (DOR), and their 95% CI. Simultaneously, we generated summary receiver operator characteristic (SROC) curves and calculated the area under the curve (AUC).[16] The sensitivity, specificity, positive and negative predicted value, and DOR of miR-203 were presented as forest plots. Moreover, the heterogeneity between the studies caused by threshold effect was quantified using Spearman correlation analysis.[17] The nonthreshold effect was assessed by the Cochran-Q method and the test of inconsistency index (I^2), and a low P-value (<0.1) and high I^2 value ($>50\%$) suggest presence of heterogeneity

caused by nonthreshold effect. If the $P<0.1$ and $I^2>50\%$, the random effect model was used, If $P>0.1$ and $I^2<50\%$, the fixed-effect model was used.[18] If the nonthreshold effect existed, meta-regression would be used to identify the sources. Fagan's nomogram and the likelihood matrix for a diagnostic test were done using Stata 12.0.[19] The Deeks' funnel plot method was applied for publication bias test.[20]

HR was adopted for prognostic evaluation in the current meta-analysis because all of the included studies used HR to measure the prognostic performance of miR-203. Study-specific HR estimates were pooled using a fixed effects model if there was no significant heterogeneity. Otherwise, a random effects model was applied. The extent of heterogeneity across studies was evaluated using χ^2 and I^2 tests; $P<0.10$ and/or $I^2>50\%$ indicates significant heterogeneity.[21] Begg[22] funnel plots and Egger linear regression tests were used to assess publication bias.[23] miR-203 expression values were divided with the highest tertile classified as high and the lower two tertiles defined as low. Publication bias and sensitivity analyses[24] were conducted using Stata 12.0 software. A P-value <0.05 was considered statistically significant.

Results
Study selection and characteristics
After initial searches of PUBMED, EMBASE, and OVID, 76 articles were retrieved. A total of 11 articles[8,25–34] were identified as eligible studies. The selection process is shown in Figure 1A, and the characteristics of the relevant studies
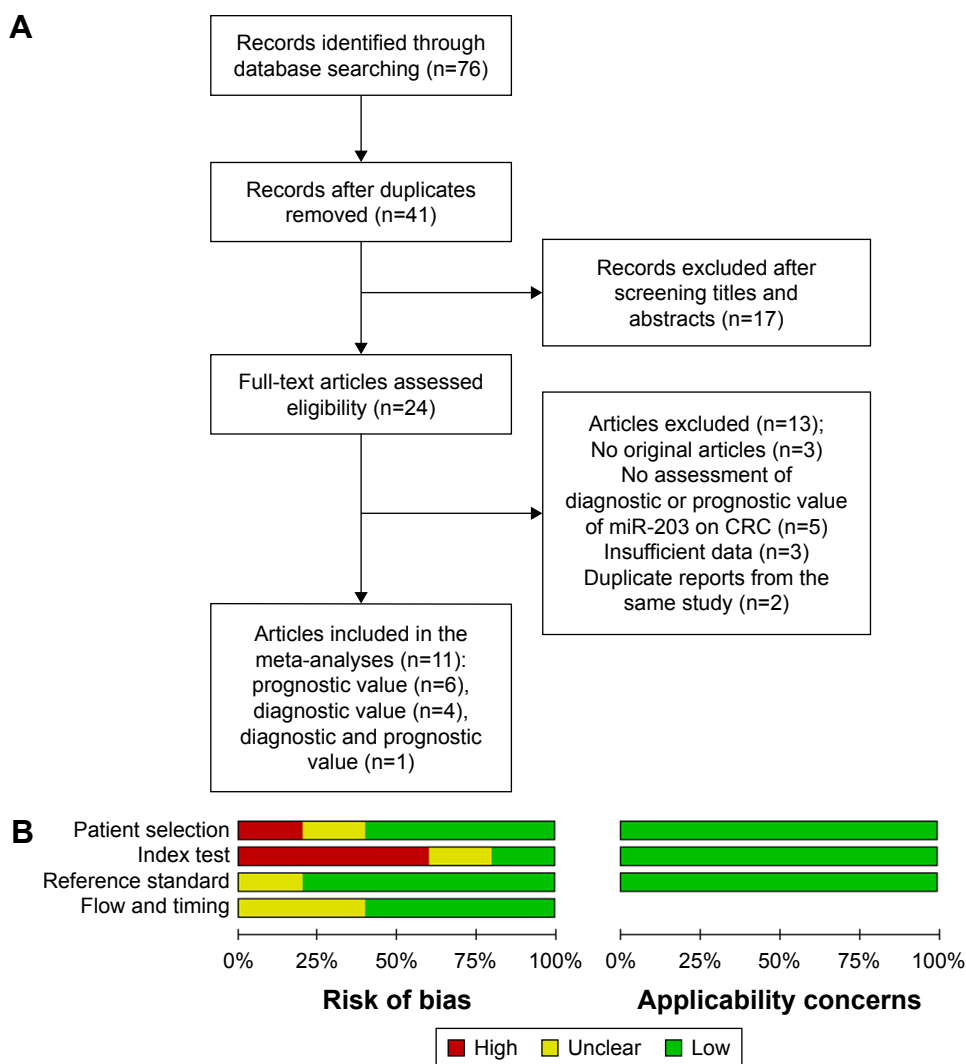

Figure 1 The flowchart depicts the selection of studies for the meta-analysis and quality assessment.
Notes: (**A**) The flowchart; (**B**) Quality assessment of the included studies for diagnostic analysis by QUADAS-2. It summarized "risk of bias" and "applicability concerns" through judging each domain for each included study. It shows the major biases concentrated upon the "index text".
Abbreviations: CRC, colorectal cancer; QUADAS-2, Quality Assessment of Diagnostic Accuracy Studies 2.

are presented in Tables 1 and 2. Among the included articles, seven reported the prognostic value of miR-203, whereas five examined diagnostic value of miR-203 (one article reported both prognostic and diagnostic value).

Diagnostic value of miR-203 for CRC

Nine independent research data from five studies with 672 total CRC patients and 261 healthy people assessed the diagnostic value of miR-203 level for CRC, from serum specimen samples. All studies used quantitative real-time polymerase chain reaction (qRT-PCR) to measure the expression of miR-203. The quality assessments are shown in Figure 1B. Since data in three independent studies from Sheinerman et al[34] were derived from the same patient, we first combined them (Figure 2A). In order to assess the heterogeneity of miR-203 among the eligible studies, we first calculated the correlation coefficient and *P*-value between the logit of sensitivity and logit of 1-specificity using Spearman test to exclude the threshold effect. The resulting Spearman correlation coefficient was −0.357 and the *P*-value was 0.432, indicating that there was no heterogeneity from threshold effect. Because of potential heterogeneity caused by nonthreshold effect among these studies, the random effect model was used to estimate overall performance of miR-203. For miR-203, the sensitivity, specificity, PLR, NLR, and DOR of included studies were evaluated by forest plots. The pooled sensitivity and specificity were 0.83 (95% CI: 0.78–0.86) and 0.80 (95% CI: 0.77–0.83), respectively (Figure 2B and C). PLR and NLR were 4.05 (95% CI: 2.60–6.29) and 0.24 (95% CI: 0.14–0.44) (Figure 2D and E). The summary DOR (Figure 2F) and the area under SROC (Figure 3) were 19.27 (95% CI: 7.23–51.36) and 0.89 (95% CI: 0.85–0.91), indicating miR-203 has a relatively high diagnostic performance in CRC.

The prognostic meta-analyses

A total of seven independent studies were included in the prognostic analyses (Table 2). All were published in English and conducted in China (n=2), Japan (n=1), or the United States (n=4). Each study assessed 67–381 patients with CRC. The types of specimen included solid tissue (n=6) and serum (n=3) (Table 3). All studies used qRT-PCR to measure miR-203 expression. The quality assessments are shown in Table 2. Six studies with 848 total patients assessed the relationship between tissue miR-203 expression and CRC OS. The pooled HR was 1.63 (95% CI: 1.03 to 2.57) for six studies from tissue, indicating that higher tissue miR-203 expression levels predicate poorer

Table 1 Main characteristics of studies included in meta-analysis for diagnosis

Study	Country	Patients/controls	Mean or median age (years)	Assay type	Internal control	Cut-off values	SE[a]	SP[b]	AUC[c]	QUADAS-2	Expression
Hur et al,[8] 2017	America	184/24	>60	qRT-PCR, $2^{-\Delta\Delta ct}$	U6 snRNA	–	47.50%	85.52%	0.678	5	Upregulated
Sun et al,[32] 2016	People's Republic of China	99/88	55	qRT-PCR, $2^{-\Delta\Delta ct}$	U6 snRNA	–	74.70%	71.40%	0.757	4	Upregulated
Wang et al,[33] 2014	People's Republic of China	30/30	>55	qRT-PCR, $2^{-\Delta\Delta ct}$	miR-16	9.595	85.29%	96.15%	0.9	4	Downregulated
Fu et al,[31] 2016	People's Republic of China	122/30	–	qRT-PCR, $2^{-\Delta\Delta ct}$	RNU6B	0.0384	83.30%	71.30%	0.78	4	Downregulated
Sheinerman et al,[34] 2013	America	10/30	57.4	qRT-PCR, $2^{-\Delta\Delta ct}$	miR-30e-3p	–	94.00%	90.00%	0.98	5	Upregulated
Hur et al,[8] 2017	America	144/24	>60	qRT-PCR, $2^{-\Delta\Delta ct}$	U6 snRNA	–	62.50%	77.68%	0.748	5	Upregulated
Wang et al,[33] 2014	People's Republic of China	83/59	>55	qRT-PCR, $2^{-\Delta\Delta ct}$	miR-16	9.595	94.55%	91.95%	0.923	4	Downregulated
Sheinerman et al,[34] 2013	America	10/30	57.4	qRT-PCR, $2^{-\Delta\Delta ct}$	miR-145	–	94.00%	86.00%	0.97	5	Upregulated
Sheinerman et al,[34] 2013	America	10/30	57.4	qRT-PCR, $2^{-\Delta\Delta ct}$	miR-148a	–	79.00%	67.00%	0.9	5	Upregulated

Notes: [a]Sensitivity. [b]Specificity. [c]The area under the curve. '–' indicates literature did not give relevant data.
Abbreviations: AUC, area under the curve; qRT-PCR, quantitative real-time polymerase chain reaction; QUADAS-2, Quality Assessment of Diagnostic Accuracy Studies 2; SE, sensitivity; SP, specificity.

Table 2 Main characteristics of the studies included in meta-analysis for prognosis

Study	Origin of population	Study design	Number of patients (M/F)	Age (years)	Stage	miR-203 assay	Cut-off	Survival analysis	Follow-up months	HR	MSQA
Deng et al,[25] 2016	People's Republic of China	R	72 (39/33)	>50	I–IV	qRT-PCR	Median	OS	84	SC	8
Hur et al,[8] 2017	America	R	Tissue 154 (90/64); Serum186 (107/79)	>60	I–IV	qRT-PCR	Median	OS	72	DE	9
Tao et al,[26] 2014	People's Republic of China	R	90 (47/43)	70	–	qRT-PCR	Median	OS	27 (3–85)	DE	8.7
Bovell et al,[27] 2013	America	R	381 (188/193)	>65	I–IV	qRT-PCR	Median	OS	Blacks 228 (84–377), Whites 180 (84–360)	DE	8
Croce et al,[30] 2013	America	R	67	>50	I–IV	qRT-PCR	Median	OS	–	DE	7.7
Toiyama et al,[29] 2012	Japan	R	120	–	I–IV	qRT-PCR	Median	OS	–	DE	7.7
Schetter et al,[28] 2008	America	R	84 (66/18)	64.6	I–IV	qRT-PCR	Median	OS	68	DE	8

Notes: Study design is described as retrospective (R). '–' indicates literature did not give relevant data.
Abbreviations: DE, data extrapolated; HR, hazard ratio; MSQA, mean score of quality assessment; OS, overall survival; qRT-PCR, quantitative real-time polymerase chain reaction; SC, survival curve.

OS (P=0.04) (Figure 4B). Significant heterogeneity across studies was observed (I^2=62%, P=0.02; Figure 4B). Three studies comprising 378 patients evaluated CRC OS for serum miR-203. We found a nonsignificant association between serum miR-203 expression level and OS (pooled HR, 1.59; 95% CI: 0.31 to 8.12, P=0.58; Figure 4C). There was significant heterogeneity in the analysis for serum miR-203 (I^2=94%, P<0.05; Figure 4C). In addition, nine studies data explored the performance of tissue and serum miR-203 levels in the prognosis of colorectal cancer. The pooled HR of nine studies for OS was 1.62 (95% CI: 0.93 to 2.82), which demonstrated the combined analysis of both tissue and serum miR-203 may have no effect on OS (P=0.09; Figure 4A).

Diagnostic test and prognostic sensitivity analysis

The Fagan's nomogram describes how to use diagnostic findings from the miR-203 assay to calculate posttest cancer probability (Figure S1A), and the overall distribution of diagnostic studies is summarized in the likelihood matrix in Figure S1B. For OS, Metainf[46] was used to investigate the influence of each study on the overall meta-analysis summary estimate, and Figure S2 shows that the results of the meta-analysis did not change after the removal of any one paper.

Publication bias

Funnel plot tests of the diagnostic and prognostic meta-analyses indicated no significant publication bias in this study (Figure 5). The Deeks funnel plot test (Figure 5A) for diagnostic value gave a P-value of 0.80. Egger (Figure 5B) and Begg (Figure S3A) tests for prognostic value of the combined analysis, which had both tissue and serum miR-203, provided P-values of 0.113 and 0.048, respectively. Moreover, for prognostic value of serum miR-203, P-values of Egger (Figure 5C) and Begg (Figure S3B) tests were 0.417 and 0.260. However, because of the limited number of included studies, publication bias cannot absolutely be ruled out.

Discussion

Although significant progress has been achieved in the diagnosis and prognosis of CRC over the years, development of better biomarkers is still necessary for early detection and for predicting patient outcomes.[35] The application of miRNAs as biomarkers for cancer diagnosis and prognosis has gained much attention in recent
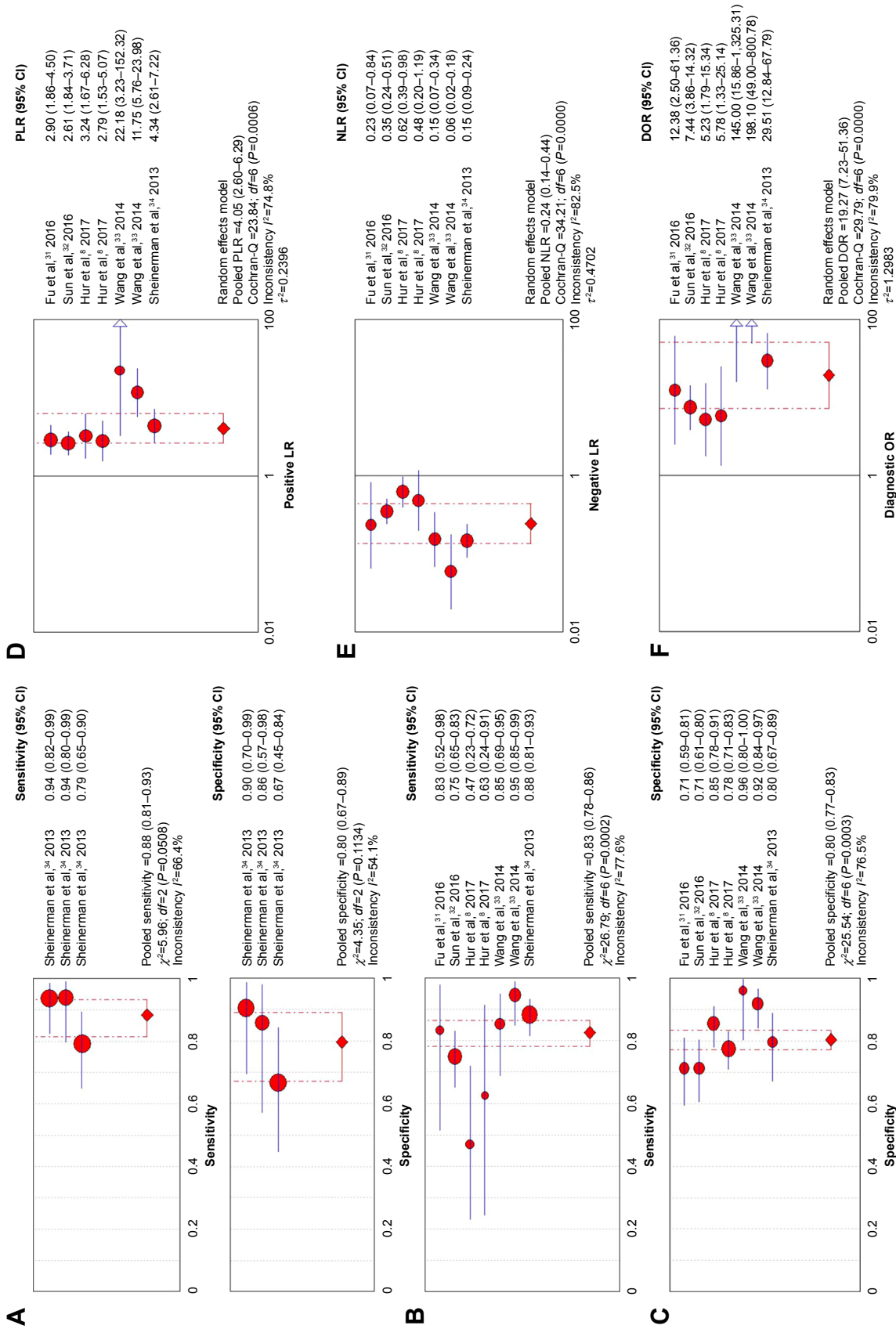
Figure 2 The forest plots show the pooled diagnosis index of miR-203 for the diagnosis of CRC.

Notes: The point efficiencies from each study are shown as circle and the pooled efficiencies are shown as diamond. Inconsistency is used to quantify the heterogeneity caused by nonthreshold effect. For these studies, random effects model was used to pool the data. (**A**) The pooled sensitivity and specificity for Sheinerman et al;[34] (**B**) The pooled sensitivity for all the included studies; (**C**) Specificity; (**D**) PLR; (**E**) NLR; (**F**) DOR, and their 95% CI are displayed respectively, which suggests miR-203 might be a potential diagnosis biomarker of CRC.

Abbreviations: CI, confidence interval; CRC, colorectal cancer; DOR, diagnostic odds ratio; NLR, negative likelihood ratio; PLR, positive likelihood ratio.
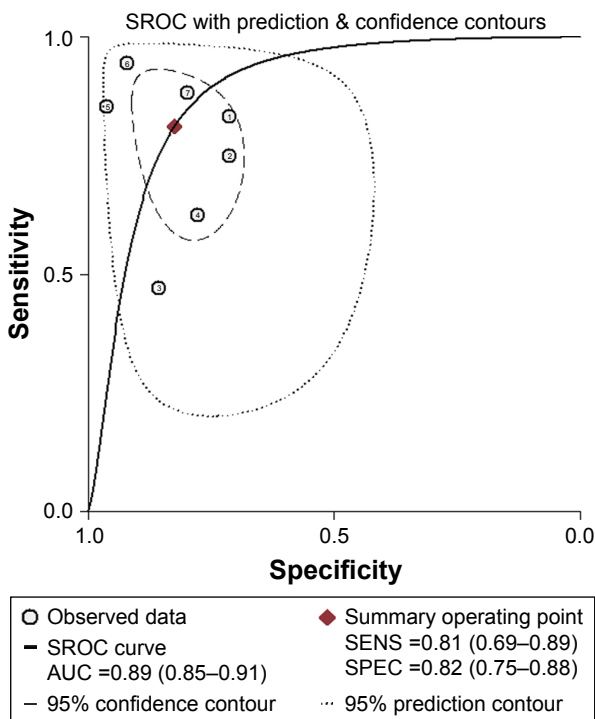
Figure 3 The SROC of miR-203 for the diagnosis of CRC.
Notes: Every circle stands for a study, the SROC curve is symmetric and the AUC is 0.89, which is consistent with moderate diagnostic accuracy for diagnosing CRC.
Abbreviations: AUC, area under the curve; CRC, colorectal cancer; SENS, sensitivity; SPEC, specificity; SROC, summary receiver operating characteristic.

years.[36,37] miR-203 is one of the most studied miRNAs to be a potential biomarker for diagnosis and prognosis of CRC. To examine the reported diagnostic and prognostic accuracies, we performed this systematic review on 11 diagnostic or prognostic studies.

miRNAs demonstrate the same change in expression in plasma, serum, feces, and tumor tissues of patients with various types of cancer.[38,39] Studies in human cancer cell lines showed that miR-203 could target tumor suppressor genes, such as *CDK6*,[40] *AKT2*,[41] *Survivin*,[42] and *ZNF217*.[43] Therefore, miR-203 may be involved in the critical steps

in carcinogenesis and progression of human cancer by promoting tumor growth, proliferation, antiapoptotic mechanisms, and migration. Moreover, Li et al[44] reported that miR-203 inhibits TYMS to improve colorectal cancer sensitivity to chemotherapy, but Zhou et al[45] showed that miR-203 can inhibit ATM kinase to reduce the sensitivity of colorectal cancer cells to chemotherapeutic drugs. These findings suggest a vital role for altered miR-203 expression in tumorigenesis.

In the present meta-analysis, miR-203 had a pooled sensitivity of 0.83, specificity of 0.80, and AUC of 0.89, suggesting that measuring miR-203 level is a promising noninvasive method for CRC diagnosis. DOR combines the strengths of both sensitivity and specificity and was reported to be a useful indicator for evaluation of the diagnostic method. The DOR value of miR-203 was 19.27, indicating a moderate diagnostic accuracy. However, the positive LR (4.05) and negative LR (0.24) suggest that miR-203 may not be adequate to distinguish patients with CRC. We found significant heterogeneity in sensitivity, and different cutoff values of miR-203 expression across studies may be one source of heterogeneity. Measuring serum miR-203 might also be a useful screening method for advanced colorectal adenomas. Serum miR-203 was also developed as a noninvasive diagnostic biomarker for CRC. On the other hand, the meta-analyses indicated that tissue miR-203 expression was a promising biomarker to predict OS in patients with CRC. Compared with patients with low miR-203 expression from tissue, patients with an increased level of miR-203 expression had a 1.63-fold higher risk of poor OS. Our results showed that miR-203 was useful to predict OS only in male participants, suggesting that sex may modify the observed effect. There was significant heterogeneity in the meta-analyses of the data for OS. Although sensitivity and subgroup analyses were applied, the heterogeneity might result from the different evaluating methods for miR-203.

Table 3 Summary table of HRs and their 95% CI

Study	HR	95% CI (LL–UL)	*P*-value	Outcome	Origin	Expression
Bovell et al,[27] 2013	1.9	1.05–3.43	0.002	OS	Tissue	Upregulated
Croce et al,[30] 2013	3.2	1.1–9.4	0.03	OS	Tissue	Upregulated
Deng et al,[25] 2016	0.62	0.32–1.2	0.0006	OS	Tissue	Downregulated
Deng et al,[25] 2016	0.36	0.20–0.64	0.0001	OS	Serum	Downregulated
Hur et al,[8] 2017	1.56	0.80–3.05	0.1962	OS	Tissue	Upregulated
Hur et al,[8] 2017	4.47	2.10–9.5	0.0001	OS	Serum	Upregulated
Schetter et al,[28] 2008	3.1	1.5–6.4	0.003	OS	Tissue	Upregulated
Tao et al,[26] 2014	1.38	0.77–2.47	0.273	OS	Tissue	Upregulated
Toiyama et al,[29] 2012	2.6	1.17–5.77	0.019	OS	Serum	Upregulated

Abbreviations: CI, confidence interval; HR, hazard ratio; LL, lower limit; OS, overall survival; UL, upper limit.
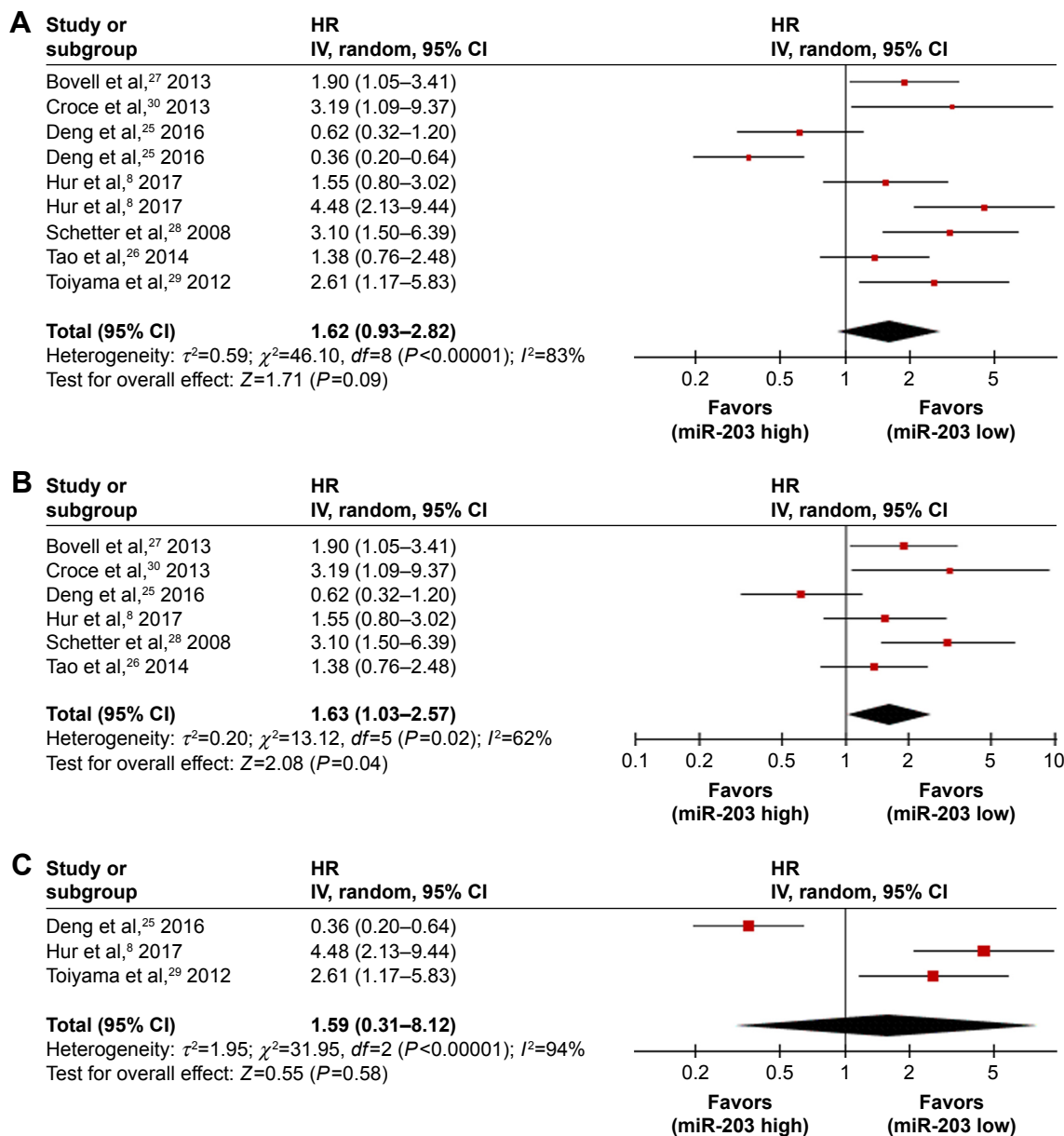
Figure 4 Forest plots of studies evaluating HRs of high miR-203 level.
Notes: (**A**) The nine survival data sets from CRC tissue and serum samples were pooled to calculate OS. The random effects analysis model showed the pooled HR for OS is 1.62 with 95% CI: 0.93–2.82, and *P*=0.09. (**B**) The six survival data sets from CRC tissue studies. The random effect analysis model was used to calculate the pooled HR, and HR =1.63 (95% CI: 1.03–2.57, *P*=0.04) for OS. (**C**) The three survival data sets from CRC serum. The random effect analysis model was used to calculate the pooled HR, and HR =1.59 (95% CI: 0.31–8.12, *P*=0.58) for OS.
Abbreviations: CI, confidence interval; CRC, colorectal cancer; HR, hazard ratio; OS, overall survival.

And, although studies have indicated that higher tissue miR-203 levels are associated with poorer OS for CRC, two kinds of samples are used for assessing miR-203 expression, from tissues and serum. To draw convincing conclusions on the value of miR-203 for the diagnosis and prognosis of CRC, an appropriate and unified method should be established and applied.

This systematic review had several important strengths. We conducted a relatively thorough systematic search and

applied a comprehensive analytic approach to evaluate the diagnostic and prognostic value of miR-203 in patients with CRC. There was considerable heterogeneity for both the diagnostic and prognostic meta-analyses. Meta-regression and subgroup analyses were applied, but the results could not fully explain the observed heterogeneity. Taken together, we conclude that serum miR-203 level is a useful biomarker for CRC detection, and tissue miR-203 is a promising marker for CRC prognosis.
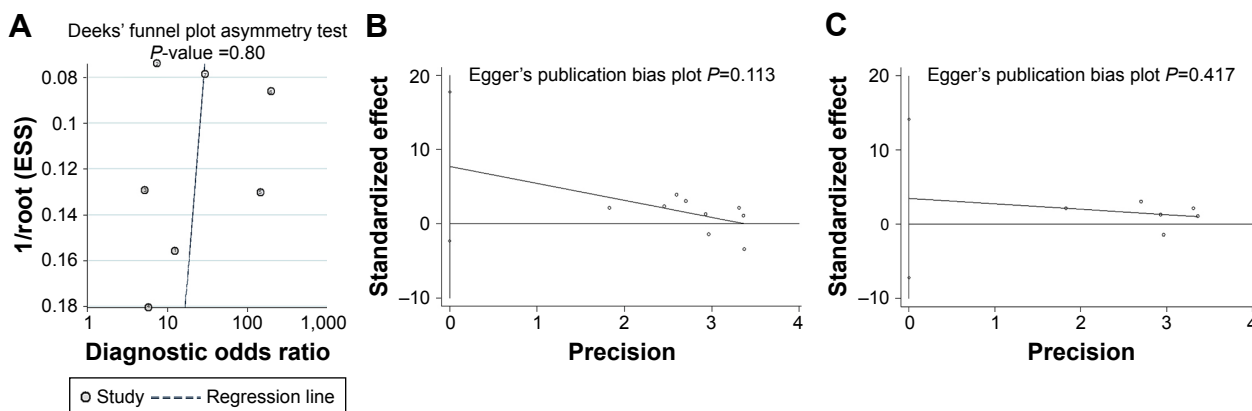
Figure 5 Publication bias based on the eligible studies for diagnosis and prognosis.
Notes: Every point represents one study, and the line is the regression line. They show no publication bias exists. (**A**) Publication bias from Deeks' test is shown by funnel plots for miR-203 diagnostic value; (**B**) Publication bias from Egger's test is shown by funnel plots for tissue and serum miR-203 prognostic value; (**C**) Publication bias from Egger's test is shown by funnel plots for serum miR-203 prognostic value.
Abbreviation: ESS, effective sample size.

Acknowledgments

Disclosure

The authors report no conflicts of interest in this work.

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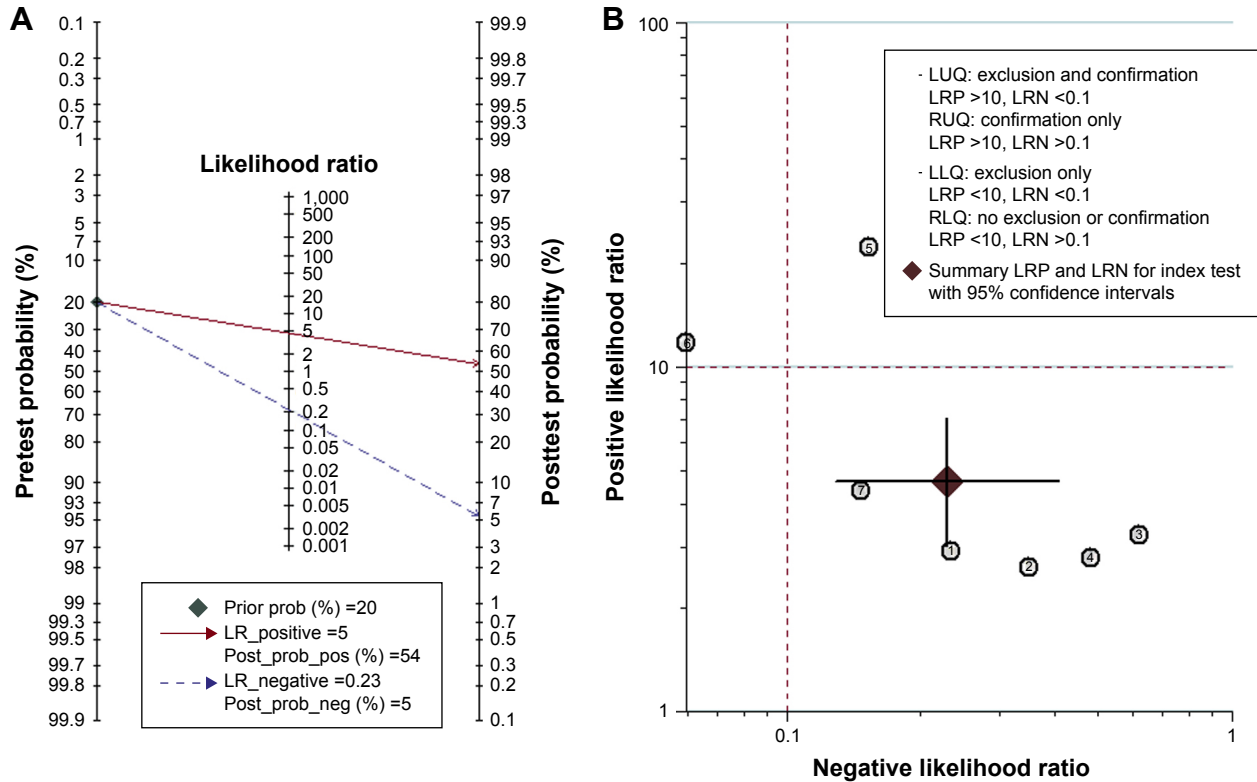
Supplementary materials

Figure S1 miR-203 diagnostic analysis based on the eligible studies.

Notes: (**A**) Fagan's nomogram describes the possibility of miR-203 assay to confirm or exclude cancer patients. In detail, for any people with a pretest probability of 20% to have cancers, if the miR-203 test in cancer detection was positive, the posttest probability to have cancer would rise to 54%; while a negative result of miR-203 assay meant the posttest probability would drop to 5% for the same people. Hence, miR-203 assay may play an important role as an initial screening method for cancer. (**B**) The overall distribution of studies is summarized in the likelihood matrix. Each corresponds to a study. ⑦, Sheinerman et al[34] was on the bottom left side of the matrix, indicating a sensitive "rule out" test. However, it reported reasonable sensitivity with incorporation bias from knowledge of a desaturation study outcome.

Abbreviations: RUQ, upper right quadrant; LUQ, upper left quadrant; RLQ, lower right quadrant; LLQ, lower left quadrant; LRN, negative likelihood ratio; LRP, positive likelihood ratio; NLR, negative likelihood ratio; PLR, positive likelihood ratio; prob, probability.
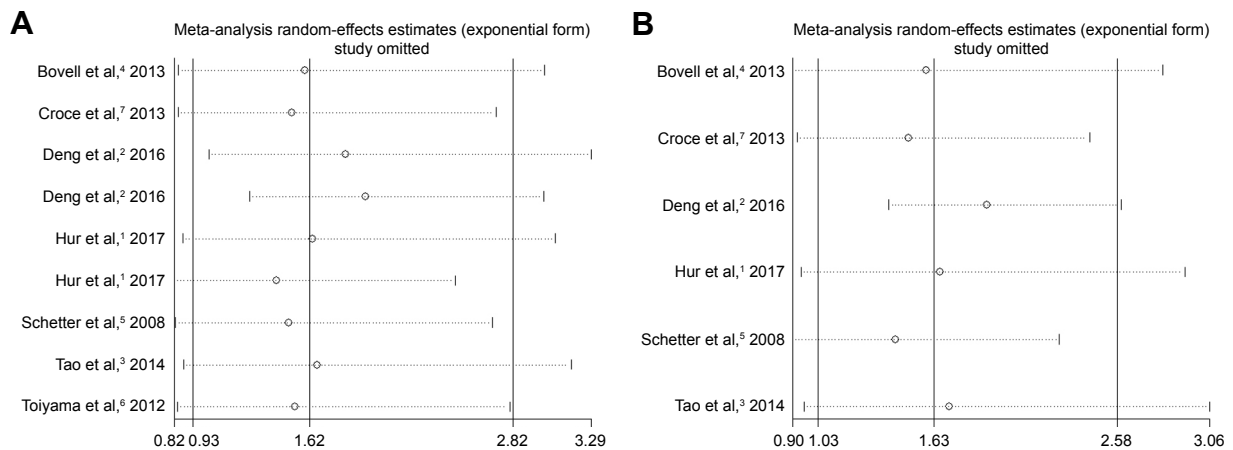

Figure S2 The sensitivity analysis based on the studies for prognosis of OS.

Notes: Forest plot for the sensitivity analysis shows the results of the meta-analysis did not change after the removal of any one paper. (**A**) The nine survival data from CRC tissue and serum; (**B**) six survival data from CRC serum.

Abbreviations: CRC, colorectal cancer; OS, overall survival.

A Begg's funnel plot with pseudo
95% confidence limits *P*=0.048

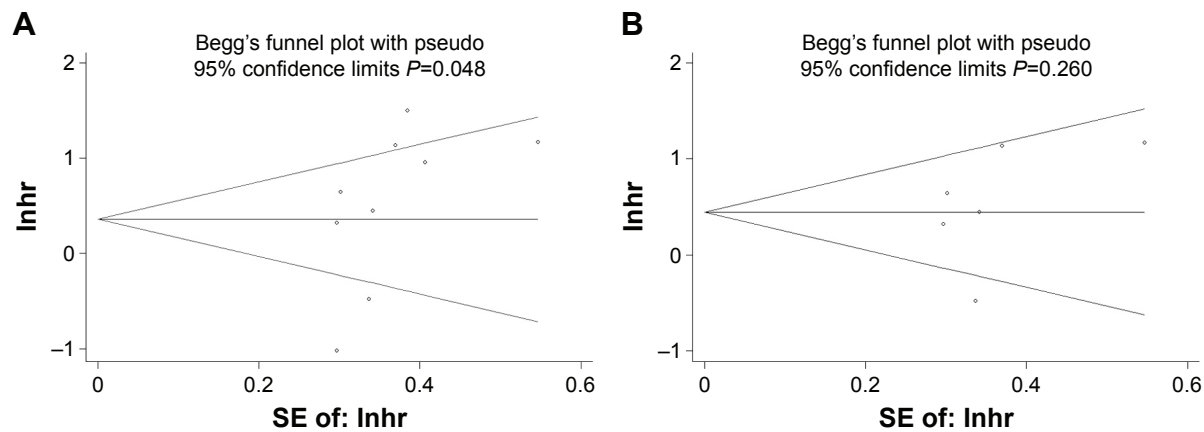**B** Begg's funnel plot with pseudo
95% confidence limits *P*=0.260

Figure S3 The publication bias based on the studies for prognosis of OS.
Notes: Publication bias from Begg's test is shown by funnel plots. Every point represents one study. (**A**) The nine survival data from CRC tissue and serum; (**B**) six survival data from CRC serum.
Abbreviations: CRC, colorectal cancer; lnhr, ln hazard ratio; OS, overall survival; SE, standard error.

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