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Original Research

MicroRNA-200 as potential diagnostic markers for colorectal cancer: meta-analysis and experimental validation

Zongyu Peng[1], Wenru Zhu[2], Jinzhao Dai[2], Fang Ju[3*]

[1] Department of Healthcare, Qingdao Cancer Hospital, The Second Affiliated Hospital of Qingdao University Medical College, Qingdao266042, Shandong, China
[2] Department of Nuclear Medicine, Qingdao Central Hospital, The Second Affiliated Hospital of Qingdao University Medical College, Qingdao266042, Shandong, China
[3] Department of Oncology, Qingdao Central Hospital, The Second Affiliated Hospital of Qingdao University Medical College, Qingdao266042, Shandong, China

Correspondence to: jufangjufang002@126.com

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Abstract: Members of microRNA(miR)-200 family is proposed as promising biomarkers for colorectal cancer (CRC). However, their expression in CRC patients, and whether them could identify as new biomarkers of cancers are inconsistent and controversy. Therefore, a meta-analysis was performed to assess the diagnostic value of miR-200 family members in CRC patients. This meta-analysis screened 6 studies, including 191 patients with colorectal cancer at stage IV, 446 patients with colorectal cancer at stage I~III and 98 normal controls, and performed using bivariate and hierarchical summary receiver operating characteristic (HSROC) models. The quality of the eligible studies was assessed according to Quality Assessment of Diagnosis Accuracy Studies-2. The pooled sensitivity and specificity of miR-141 alone for CRC diagnosis were 82% and 75%, respectively. The diagnostic odds ratio (DOR) value was 13.21 [95% confidence interval (CI), 7.00-24.95], and the area under the curve (AUC) was 0.85 (95% CI, 0.82-0.88). The pooled sensitivity and specificity of total miR-200 family members were 79% and 71%, respectively. In the HSROC model, the estimate for the "Lambda" was 2.48 (95% CI,1.50-3.46). Finally, we detected the miR-141 in 20 CRC patients and 20 healthy. Results showed that serum miR-141 was overexpressed in CRC patients. Overall, miR-141 in miR-200 family has a good sensitivity and moderate specificity for CRC diagnosis.

Key words: miR-141; miR-200b, miR-200c; Colorectal cancer; Diagnostic.

Introduction

Colorectal cancer (CRC) is one of the most commonly malignancies worldwide, and its mortality was remained high. On the one hand, the metastasis had occurred when the patient was first diagnosed (1, 2). In present, early detection of CRC are based on traditional screening methods, such as the fecal occult blood test (FOBT) (3, 4). The high mortality of CRC was due to the late diagnosis. On the other hand, it was lack of an effective therapy (1, 5, 6). How to find a noninvasive biomarker that can detect CRC with high precision in CRC early progression was important (7-9).

MicroRNAs (miRNAs, miR) are small non-coding RNA, with nucleotides range from 18-25 (10). They regulate translation of sequence-specific genes through binding to mRNA 3 'untranslated area targets (11, 12). It was reported that miRNA plays important roles in different cell processes that involved in human diseases, including cell growth, differentiation, invasion, angiogenesis, and epithelial-mesenchymal transition (13, 14). MiR-200b, miR-200c, and miR-141 belonging to miR-200 family were tumor suppressor genes that widely participated in occurrence and development of various cancers (15, 16).

Previous study revealed that miR-200b and miR-200c significantly declined in breast cancer and gastric cancer (17, 18), whereas miR-200b markedly reduced in colorectal cancer tissue compared with normal control (19, 20). In this study, a meta-analysis was performed to assess the diagnostic value of miR-200 family members in CRC patients.

Materials and Methods

Literature Search Strategy

Articles published up to January 23, 2018. Relevant studies were identified by searching PubMed, EMBASE, Web of Science, China National Knowledge Infrastructure (CNKI), and Cochrane Library. The search strategy was (colorectal AND (cancer OR tumor OR neoplasms OR carcinoma)) AND (microrna AND 200 OR (microrna AND 429) OR (microrna AND 141). In addition, we also examined the reference lists in identified articles to find any additional relevant studies. Two investigators (PZ and ZW) independently carried out the literature search and the following tasks.

Selection criteria

Two reviewers (ZW and DJ) independently assessed the literature extracted by the search strategy. Whenever they had different opinions, the reviewers discussed

until a consensus was reached. The criteria were as follows: Selection criteria:(1) patients with colorectal cancer were confirmed by pathology (gold standard);(2) include miR-200 family members such as miR-200a/200b/200c/429/141 for the diagnosis of colorectal cancer alone: sensitivity or specificity;(4) the control group was set up;(5) a similar literature published by the same author or research center, which is included in the recent literature published or influential factors;(6) the number of cases in each group was greater than 10;(7) nationality and race, and publish in Chinese or English. Exclusion criteria :(1) repeated articles or studies;(2) data deletion cannot be quantitatively synthesized;(3) non-human studies, cellular studies, reviews, summary of meetings, or case reports.

Eligibility criteria

The main criteria considered for the enrollment of studies were as follows: (1) they reported research on patients with CRC; (2) they detected miR-200a/200b/200c/141/429 expression in plasma, serum, feces, or tissues; (3) they made a definitive diagnosis of CRC with the gold standard; (4) they provided sufficient data for calculating the rates of true positive (TP), false positive (FP), false negative (FN), and true negative (TN) for diagnostic meta-analysis. Studies were excluded if they were (1) not relevant to our study topic; (2) published in the form of letters, reviews, editorials, or case reports; (3) duplicate publications; or (4) involved unqualified data.

Data extraction and quality assessment

According to the selection criteria and eligibility criteria, the two researchers (PZ and WZ) independent literature selection, quality evaluation and information extraction, collected the relevant data from the articles based on standardized forms. If there are any differences, the first step was cross check, and then the parties to discuss or settled by consulting with a third reviewer (DJ) until reach a consensus. The final inclusion of the literature reference Quality Assessment of Diagnosis Accuracy Studies-2 (quadas-2) scores (21). Quadas-2 is a recognized quality evaluation tool for diagnostic tests, consisting of four domains: (1) patient selection;(2) index test;(3) reference standard;(4) flow and timing. The signature issues of each domain are judged by "high", "unclear" and "low" to judge the risk of bias and its applicability.

Patients and serum miR-141 detection

Twenty CRC patients and twenty healthy subjects were enrolled and the serum level of miR-141 were detected. Total RNA (1 μg) was reversed with RevertAid First Strand cDNA Synthesis Kits (Thermo, USA) according to the manufacturer's instructions. Real-time qPCR was performed with SYBR Green PCR kits. The primers (5'-3') were list as follow: Hsa-miR-141 stem loop primer GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCCATCT,

Hsa-miR-141 forward GGTCCTAACACTGTCTGGTAAAGTGG, and Hsa-miR-141 reverse CCAGTGCAGGGTCCGAGGT. Hsa-U6 forward TGCGGGTGCTCGCTTCGGCAGC, and Hsa-U6 reverse CCAGTGCAGGGTCCGAGGT. U6 was used as the endogenous controls. Relative fold expressions were calculated with the comparative threshold cycle ($2^{-\Delta\Delta Ct}$) method. The study was approved by the Ethics Committee of the University and was carried out according to the World Medical Association Declaration of Helsinki.

Statistical analysis

For the diagnostic meta-analysis, true positive, false positive, true negative and false negative were extracted as bivariate data directly or through recalculation on the basis of relative data from each eligible study. Through the summary into the research of data (true positive/tp and false positive/fp and true negative/tn and false negative/fn), get the overall sensitivity, specific, positive likelihood ratio and negative likelihood ratio, diagnostic odds ratio(DOR) and the corresponding 95% confidence interval (95% CI), and synthesis of total the receiver-operating characteristic curve (the summary receiver operator characteristic, SROC) and calculate the area under the curve (area under the curve, AUC). The accuracy of the results was further verified by applying the hierarchical summary receiver operating characteristics (HSROC) model and subsequently presented the HSROC curve (22). Furthermore, the HSROC curve is closely associated with the bivariate random-effect model. All the analyses were conducted by Meta-DiSc and Stata SE12.0 software (23).Values of $P < 0.05$ were deemed to represent statistical significance.

Results

Literature search strategy and included studies.

This meta-analysis was according to the standard of PRISMA statement(24), Relevant studies were identified by searching PubMed, EMBASE, Web of Science, China National Knowledge Infrastructure (CNKI), and Cochrane Library. The search terms were: (colorectal AND (cancer OR tumor OR neoplasms OR carcinoma)) AND (microrna AND 200 OR (microrna AND 429) OR (microrna AND 141)). The full search strategy is available on table 1. The last search was conducted on January 23, 2018. A total of 97 full text articles were selected for detailed evaluation following the selection criteria and eligibility criteria. Finally, 6 studies were included in the analysis including 191 patients with colorectal cancer at stage IV, 446 patients with colorectal cancer at stage I~III and 98 normal controls(25-30). The flow chart of the study selection process and detail of them is shown in Fig. 1.

Study characteristics and methodological quality assessment

In these selected studies, 637 CRC patients have been confirmed by pathology. In addition, 403 controls were healthy volunteers, paired normal tissues or CRC patients without any metastases. Enrolled in the system assessment studies were conducted in China and Italy. Of the six studies, five were conducted in Asian populations, and one was conducted among Caucasians populations. The Six studies were published between 2011 and 2017. All the studies used reverse transcription-quantitative polymerase chain reaction (RT-qPCR) to assess the expression,1 study assess the expression of miR-200b,1 study assess the expression of miR-200c,1

Table 1. Search result.

Data base	Search strategy	Numbers
PubMed	(colorectal cancer[Title/Abstract] OR colorectal tumor [Title/Abstract] OR colorectal neoplasms [Title/Abstract] OR colorectal carcinoma [Title/Abstract]) AND (miR 200[Title/Abstract] OR miR 429[Title/Abstract] OR miR 141[Title/Abstract])	13
EMBASE	(colorectal AND (cancer OR tumor OR neoplasms OR carcinoma)) AND (miR AND 200 OR (miR AND 429) OR (miR AND 141))	330
Web of Science	TI=(Colorectal AND (cancer OR tumor OR neoplasms OR carcinoma)) AND TS=((miR 200) OR (miR 429) OR (miR 141))	53
CNKI	(Title=Colorectal cancer OR Title=Colorectal tumor) AND (Abstract=miR 200 OR Abstract=miR 429 OR Abstract=miR 141)	5
Cochrane Library	#1 colorectal cancer:ti,ab,kw or colorectal carcinoma:ti,ab,kw (Word variations have been searched)	9606
	#2 miR 200:ti,ab,kw or miR 429:ti,ab,kw or miR 141:ti,ab,kw (Word variations have been searched)	25
	#3 #1 and #2	0

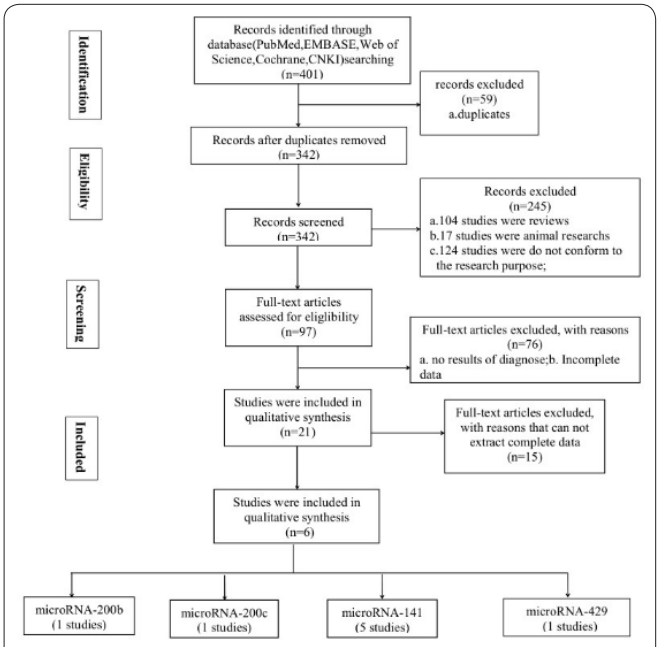

Figure 1. Flow chart of the study selection.

study assess the expression of miR-429,5 studies assess the expression of miR-141. The main characteristics of the eligible studies are presented in Table 2.

The qualified QUADAS-2(21)evaluation was included in the study. As shown in table 3, the quality of these six studies included the study of low and high risks, but the "index test" and "reference standard" was a big deviation, because the gold standard test did not find blindness; Patient selection is also biased because most studies do not report or do not include random cases.

Data analysis

Diagnostic meta-analysis of miR-141 alone in CRC

Heterogeneity in sensitivity and specificity was detected in the 5 studies (I^2=45.11%, P=0.12 and I^2=75.83%, P=0.00 respectively), suggesting mild heterogeneity in sensitivity and significant heterogeneity in specificity (Fig. 2). In the system meta-analysis, the random effects model was employed. The analysis results showed that the pooled sensitivity and specificity of miR-141 for CRC diagnosis were 82% [95% confidence interval (CI), 75-87] and 75% (95% CI, 65-83), respectively.

MiR-141 PLR and NLR were calculated for likelihood ratios. These parameters are considered more clinically valuable than specificity and sensitivity(31). PLR >10 or NLR <0.1 suggests high diagnostic accuracy. In the present study, the pooled PLR is 3.22 (95% CI, 2.23-4.66; I^2=57.80%), indicating that the case group have more than a three-fold probability to express miR-141 in comparison to control individuals. The pooled NLR was 0.24 (95% CI, 0.17-0.34; I^2=59.43%) (Fig. 3). The SROC curve of the selected studies is shown in Fig. 4. The AUC was 0.85 (95% CI, 0.82-0.88). The DOR value was 13.21 (95% CI, 7.00-24.95), indicating that miR-141 can be used as a good marker for CRC diagnosis (Fig. 5).

The HSROC curve of the selected studies is shown

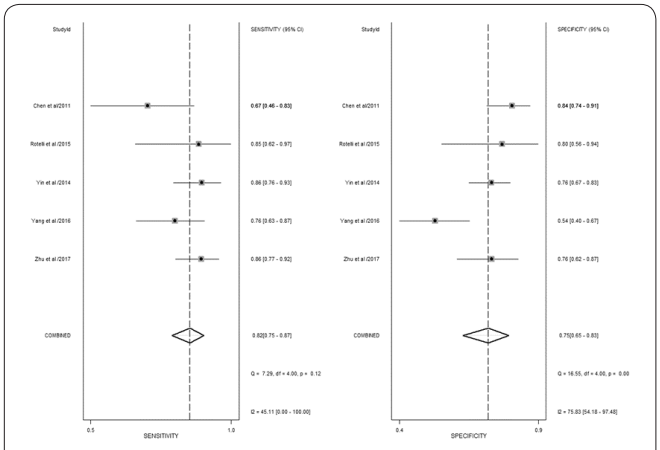

Figure 2. Forest plots of sensitivities and specificities for miR-141 test accuracy in the diagnosis of colorectal cancer.

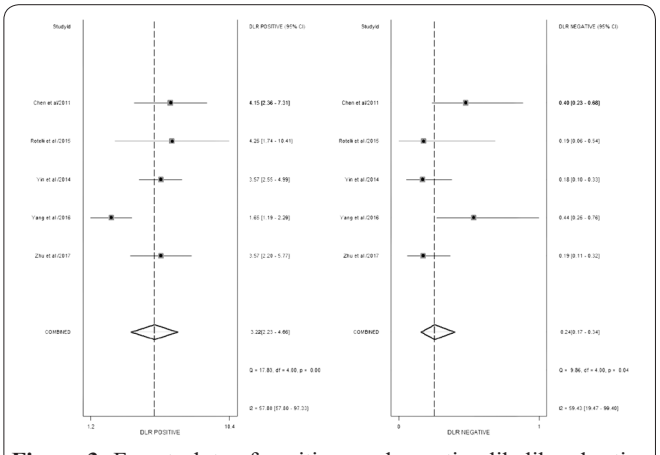

Figure 3. Forest plots of positive- and negative-likelihood ratios for miR-141 test accuracy in the diagnosis of colorectal cancer.

Table 2. Main characteristics of the eligible studies.

MiR	Author	Year	Country	Ethnicity	RNA extraction kits	Test method	Calculation method	Case,n/ Control,n	case		TNM stage		Sample	AUC	Se(%)	Sp(%)
									colon (n)	rectum,(n)	IV	I-III				
miR-141	Zhu et al	2017	China	Asian	miRNeasy RNA	RT-qPCR	$Log_{10}(2^{-\Delta\Delta Ct})$	85/54	54	31	85	54	Serum	0.83	86	76
	Yang et al	2016	China	Asian	Trizol-LS	RT-qPCR (Taqman)	$Log_{10}(2^{-\Delta\Delta Ct})$	50/54	28	22		104	Tissue	0.62	77	53
	Yin et al	2014	China	Asian	miRcute miRNA	RT-qPCR	$Log_{10}(2^{-\Delta\Delta Ct})$	72/116	48	24	72	116	Serum	0.83	86	76
	Rotelli et al	2015	Italy	Caucasian	miRNeasy RNA	RT-qPCR (SYBR-Green)	miR141/miR16* ratio	20/20	12	8		20	Feces	0.97	84	79
	Chen et al	2011	China	Asian	Trizol-LS	RT-qPCR (Taqman)	$Log_{10}(2^{-\Delta\Delta Ct})$	27/81	na	na	27	81	Plasma	0.76	67	84
miR-200b	Zhu et al	2017	China	Asian	miRNeasy RNA	RT-qPCR	$Log_{10}(2^{-\Delta\Delta Ct})$	85/54	54	31	85	54	Serum	0.76	78	69
miR-200c	Zhu et al	2017	China	Asian	miRNeasy RNA	RT-qPCR	$Log_{10}(2^{-\Delta\Delta Ct})$	85/54	54	31	85	54	Serum	0.75	74	66
miR-429	Dong et al	2016	China	Asian	Trizol-LS	RT-qPCR (SYBR-Green)	$2^{-\Delta\Delta Ct}$	78/78	37	41	7	71	Tissue	0.78	72	63

Table 3. QUADAS-2 assessment for the eligible studies.

Study	Zhu et al 2017	Yang et al 2016	Yin et al 2014	Rotelli et al 2015	Dong et al 2016	Chen et al 2011
(1) patient selection	Unclear	High risk	Low risk	High risk	High risk	Unclear
1.Was a consecutive or random sample of patients enrolled?	U	N	Y	U	N	U
2.Was a case-control design avoided?	Y	Y	Y	Y	Y	Y
3.Did the study avoid inappropriate exclusions?	Y	Y	Y	N	Y	Y
(2) index test	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
1.Were the index test results interpreted without knowledge of the results of the reference standard?	U	U	U	U	U	U
2.If a threshold was used, was it pre-specified?	U	U	U	U	U	U
(3) reference standard	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
1.Is the reference standard likely to correctly classify the target condition?	Y	Y	Y	Y	Y	Y
2.Were the reference standard results interpreted without knowledge of the results of the index test?	U	U	U	U	U	U
(4) flow and timing	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
1.Was there an appropriate interval between index test(s) and reference standard?	Y	Y	Y	Y	Y	Y
2.Did all patients receive a reference standard?	Y	Y	Y	Y	Y	Y
3.Did patients receive the same reference standard?	Y	Y	Y	Y	Y	Y
4.Were all patients included in the analysis?	Y	Y	Y	Y	Y	Y

Y, Yes; U, Unclear; N,No.

in Fig. 6, which is consistent with the results from the bivariate model. The summary operating point estimate of sensitivity and specificity is also presented. The 95% prediction and 95% CI are also plotted. The cut-off point was located near the upper left corner of the HSROC curve. In the results obtained by the HSROC model,

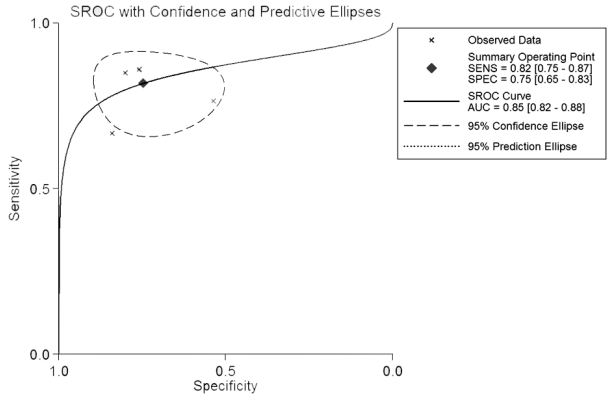

Figure 4. Summary receiver operating characteristic curves for miR-21 in the diagnosis of colorectal cancer.

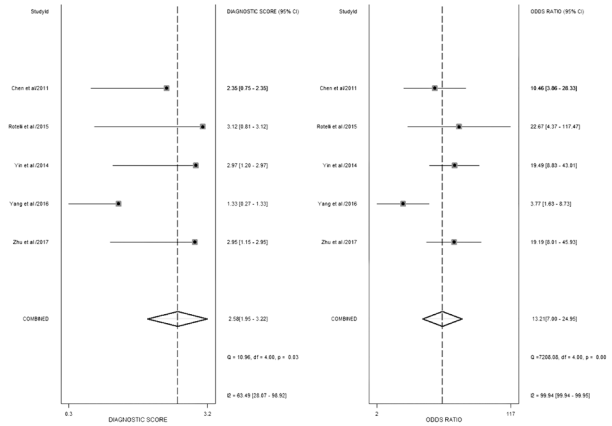

Figure 5. Forest plots of diagnostic odds ratio for miR-141 test accuracy in the diagnosis of colorectal cancer.

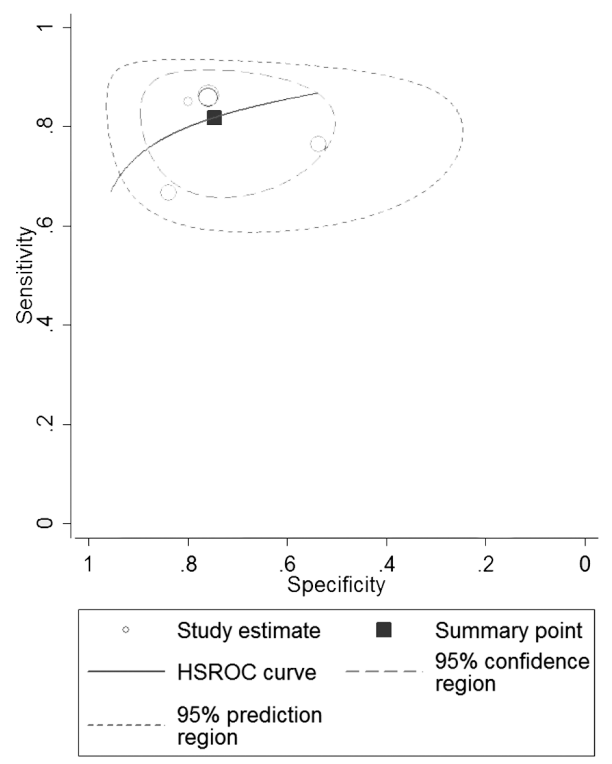

Figure 6. Hierarchical summary receiver operating characteristics (HSROC) curve for miR-141 in the diagnosis of colorectal cancer.

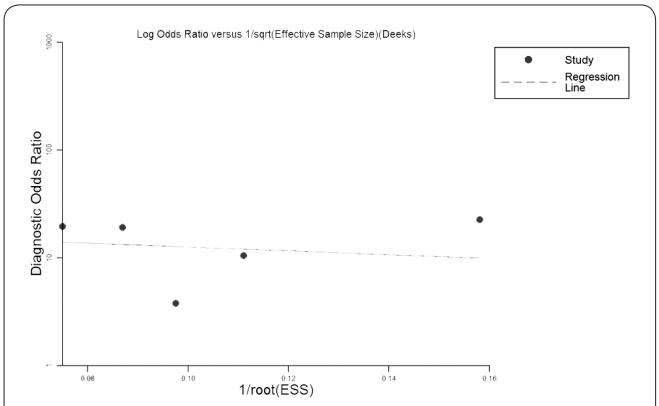

Figure 7. Deeks' tests for the assessment of publication bias in miR-141 assays.

there is a corresponding one. The "beta" estimate and its 95% confidence interval are 0.91 (-3.21,5.04), and the z-statistic is 0.43, *P* value was 0.665, prompt HSROC is symmetric; The effect of diagnostic test discrimination ability is reflected. The estimate for the "Lambda" and its 95% confidence interval was 3.05(-0.66, 6.77) suggesting that the value of the experiment needs further study.

Publication bias

Deeks' funnel plots were used to evaluate the presence of publication bias in this meta-analysis. The funnel plot presents no asymmetry (Fig. 7). The *P*-value was 0.82, indicating the absence of publication bias in the meta-analysis. However, concluding whether or not publication bias exists is difficult, due to the limited number of studies involved in the current meta-analysis.

Threshold effect and heterogeneity

Differences in cut-off values cause the threshold effect. The ROC plane and Spearman rank correlation test is a good approach to assess the threshold effect(23). In the present study, the representation of the sensitivity against the specificity of each study is shown in an ROC space (Fig. 8), which can be used to detect the threshold effect. The pattern of the points in this Fig. does not suggest a 'shoulder-arm' shape, indicating the absence of the threshold effect. A Spearman rank correlation was

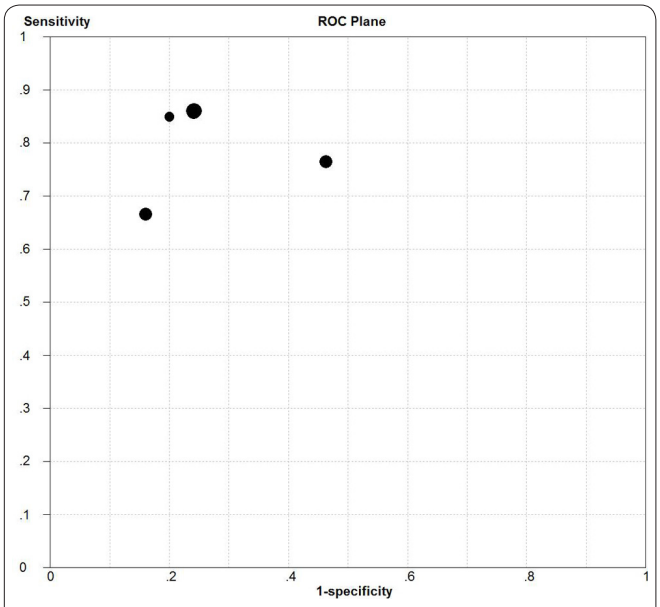

Figure 8. Receiver operating characteristics (ROC) space for the assessment of threshold effect in miR-141 arrays.

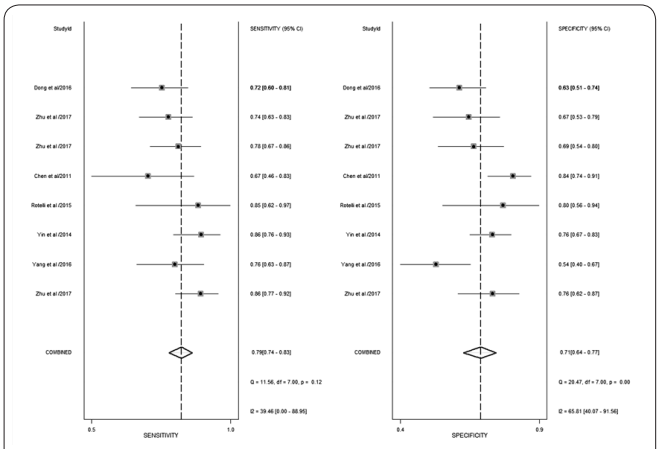

Figure 9. Forest plots of sensitivities and specificities for miR-200 family members test accuracy in the diagnosis of colorectal cancer.

conducted and the absence of heterogeneity was validated from the threshold effect [Spearman correlation coefficient=0.4; P=0.505 (P>0.05)].

The country, ethnicity, the different kinds of samples, the methods of calculation and the stage of CRC patients may cause of the heterogeneity. Meta-regression analysis suggests that the country (p=0.64), the ethnicity (p=0.64), the different kinds of sample (p=0.12), the methods of calculation (p=0.64) and the stage of CRC patients (p=0.35) are not the sources of heterogeneity in this study.

Diagnostic meta-analysis of miR-200 family members in CRC

Heterogeneity in sensitivity and specificity was detected in the 8 studies (I^2=39.46%, P=0.12 and I^2=65.81%, P=0.00 respectively), suggesting mild heterogeneity in sensitivity and significant heterogeneity in specificity (Fig. 9). In the system meta-analysis, the random effects model was employed. The analysis results showed that the pooled sensitivity and specificity of miR-200 family members for CRC diagnosis were 79% (95% CI, 74-83) and 71% (95% CI, 64-77), respectively.

MiR-200 family members PLR and NLR were calculated for likelihood ratios; these parameters are considered more clinically valuable than specificity and sensitivity (31). PLR >10 or NLR <0.1 suggests high diagnostic accuracy. In the present study, the pooled PLR is 2.75 (95% CI, 2.13-3.55; I^2=44.27%), indicating

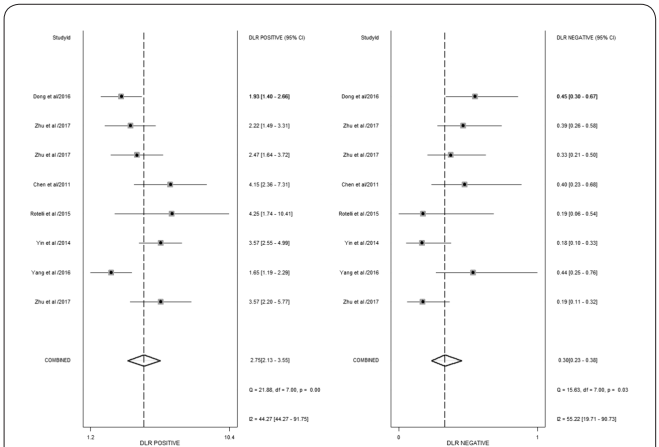

Figure 10. Forest plots of positive- and negative-likelihood ratios for miR-200 family members test accuracy in the diagnosis of colorectal cancer.

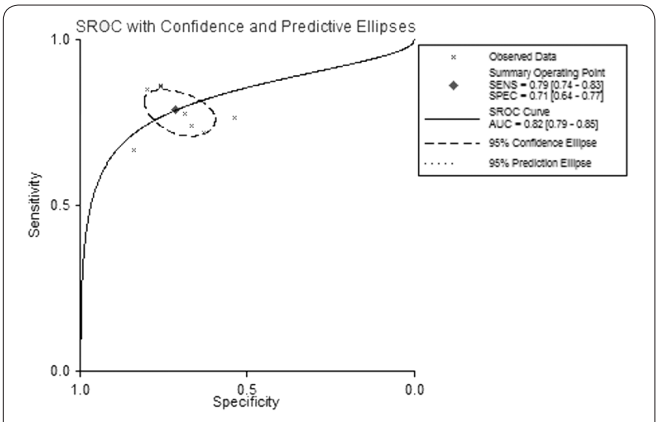

Figure 11. Summary receiver operating characteristic curves for miR-200 family members in the diagnosis of colorectal cancer.

that the case group have more than a two-fold probability to express miR-200 family members in comparison to control individuals. The pooled NLR was 0.30 (95% CI, 0.23-0.38; I^2=55.22%) (Fig. 10). The SROC curve of the selected studies is shown in Fig. 11. The AUC was 0.82 (95% CI, 0.79-0.85). The DOR value was 9.26(95% CI, 5.71-15.03), indicating that miR-200 family members can be used as a good marker for CRC diagnosis (Fig. 12).

The HSROC curve of the selected studies is shown in Fig.13, which is consistent with the results from the bivariate model. The summary operating point estimate of sensitivity and specificity is also presented. The 95% prediction and 95% CI are also plotted. The cut-off point was located near the upper left corner of the HSROC curve. In the results obtained by the HSROC model, there is a corresponding one. The "beta" estimate and its 95% confidence interval are 0.65 (-0.90,2.21), and the z-statistic is 0.82, P value was 0.409, prompt HSROC is symmetric; The effect of diagnostic test discrimination ability is reflected. The estimate for the "Lambda" and its 95% confidence interval was 2.48(1.50, 3.46), suggesting that miR-200 family members are good diagnostic marker for CRC, and miR-141 in miR-200 family was relatively accurate diagnostic marker for CRC.

Publication bias

Deeks' funnel plots were used to evaluate the presence of publication bias in this meta-analysis. The funnel plot presents no asymmetry (Fig. 14). The P-value was 0.81, indicating the absence of publication bias

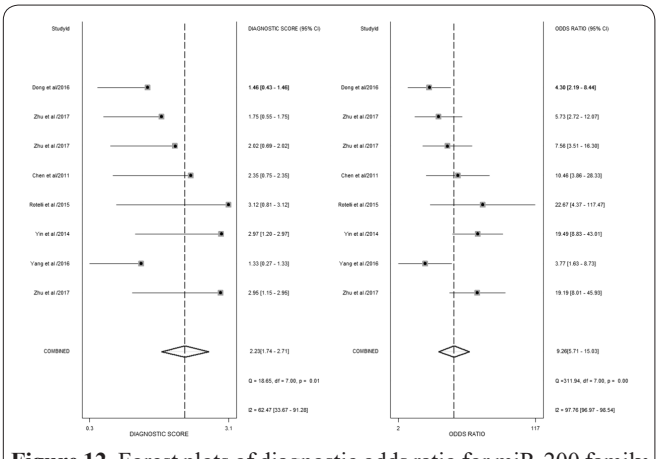

Figure 12. Forest plots of diagnostic odds ratio for miR-200 family members test accuracy in the diagnosis of colorectal cancer.

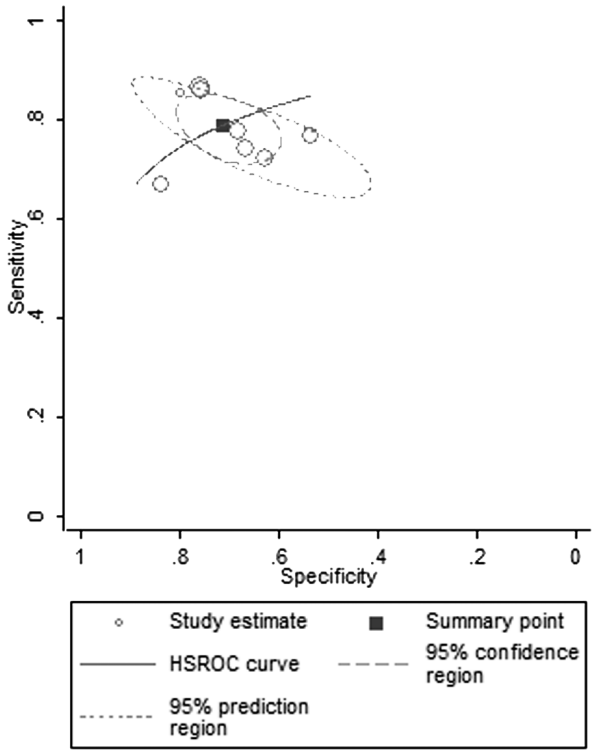

Figure 13. Hierarchical summary receiver operating characteristics (HSROC) curve for miR-200 family members in the diagnosis of colorectal cancer.

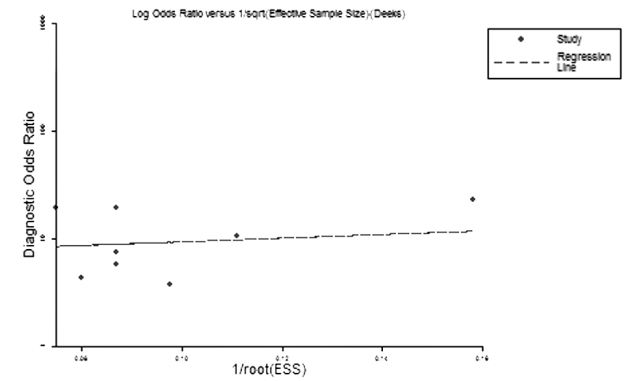

Figure 14. Deeks' tests for the assessment of publication bias in miR-200 family members assays.

in the meta-analysis. However, concluding whether or not publication bias exists is difficult due to the limited number of studies involved in the current meta-analysis.

Threshold effect and heterogeneity

Differences in cut-off values cause the threshold effect. The ROC plane and Spearman rank correlation test is a good approach to assess the threshold effect(23). In the present study, the representation of the sensitivity against the specificity of each study is shown in an ROC space (Fig. 15), which can be used to detect the threshold effect. The pattern of the points in this Fig. does not suggest a 'shoulder-arm' shape, indicating the absence of the threshold effect. A Spearman rank correlation was conducted and the absence of heterogeneity was validated from the threshold effect [Spearman correlation coefficient=-0.167; P=0.693 (P>0.05)].

The different kinds of miR-200 family members, the country, ethnicity, the different kinds of samples, the methods of calculation and the stage of CRC patients may cause of the heterogeneity. Meta-regression

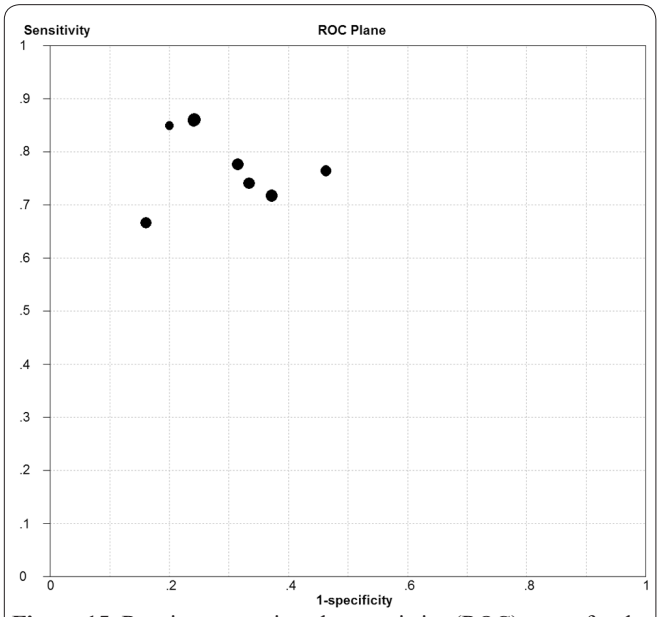

Figure 15. Receiver operating characteristics (ROC) space for the assessment of threshold effect in miR-200 family members assays.

analysis suggests that the different kinds of miR-200 family members(p=0.13), the country(p=0.39), the ethnicity(p=0.39), the different kinds of sample(p=0.69), the methods of calculation(p=0.49) and the stage of CRC patients (p=0.78) are not the sources of heterogeneity in this study.

Differential expression of miR-141 between CRC patients and healthy subjects

The serum levels in 20 CRC patients and 20 healthy subjects were detected by qRT-PCR. The serum miR-141 level in CRC patients were significantly higher than the control group (1±0.1 vs. 2.1±0.6, P<0.05).

Discussion

CRC is one of the most commonly diagnosed cancers around the world, and the mortality rate remains high, partly due to the late diagnosis (1, 4). Therefore, there is an urgent need of identification of a biomarker for early detection of CRC in routine clinical lab tests (6). In the recent years, miRs circulating in the serum have been explored for such purposes, and miR-200 family members have been proposed as promising noninvasive biomarkers for CRC (7). However, the diagnostic value of these miRs remains in controversy because of the small sample sizes in and potential inconsistencies among different individual studies (8, 16). To address this critical issue, we presently performed a meta-analysis, in which assessed the likelihood of using miR-200 family members as reliable biomarkers for CRC diagnosis by searching multiple major literature databases for studies of miR-200 members and CRC. Here, we identified six qualified studies with miRNAs measurements in samples from over 600 CRC patients and 98 normal controls, which allowed us to calculate the sensitivity and specificity for CRC diagnosis. We concluded that miR 200 family members, especially miR-141, were good markers for CRC prediction. To further strengthen our conclusion, we measured the miR-141 levels in sera of 20 CRC patients and 20 healthy controls, and results showed that miR-141 levels were indeed upregulated in

CRC.

In the present meta-analysis, we showed that the pooled sensitivity and specificity of miR-141 for CRC diagnosis were 82% and 75%, respectively, which indicates good sensitivity and moderate specificity. The DOR value was 13.21 (95% CI, 7.00-24.95), indicating that miR-141 can be used as a good marker for CRC diagnosis. The AUC was 0.85 (95% CI, 0.82-0.88), which indicates that miR-141 demonstrates good accuracy for CRC diagnosis. The HSROC curve of those selected studies showed 82% sensitivity is and 75% specificity. The estimate for the "Lambda" was 3.05 (95%CI, -0.66, 6.77), suggesting that the value of those experiments needs to be further studied in the future. MiR-141 serum levels were higher expressed in CRC patients. The analysis results showed that the pooled sensitivity and specificity of total miR-200 family members for CRC diagnosis were 79% and 71%, respectively. The estimate for the "Lambda" was 2.48 (95%CI, 1.50, 3.46). These results suggest that miR-141 in miR-200 family is relatively accurate diagnostic marker for CRC. From the perspective of sensitivity and specificity, miR-141 is superior to the entire miR-200 family; from the point of view of diagnostic test diagnostic ability, the entire miR-200 family is superior to miR-141.

The representation of the sensitivity against the specificity of each study is shown in an ROC space, which can be used to detect the threshold effect. A Spearman rank correlation was conducted and the absence of heterogeneity was validated from the threshold effect [Spearman correlation coefficient=0.4; P=0.505 (P>0.05)]. This result indicates that threshold effect is not the reason of heterogeneity. Meta-regression analysis suggests that the ethnicity and the stage of CRC patients are not the sources of heterogeneity in this study.

Deeks' funnel plots were used to evaluate the presence of publication bias in this meta-analysis. The funnel plot presents no asymmetry. The P-value was 0.82, indicating the absence of publication bias in the meta-analysis. However, concluding whether exist publication bias in this study is difficult, due to the limited number of studies involved in the current meta-analysis. In addition, miRNAs circulating in the serum are not unique to CRC. They can arise from different types of cancer. For example, elevated levels of miR-141 were detected in sera of patients with prostate cancer (32), lung cancer (33), and ovarian cancer (34). Even pregnant women have high levels of miR-141 in their sera (35). Moreover, if the miR-141 in serum will be confirmed as a unique biomarker for the early diagnosis of colorectal cancer, the collected studies should be prospective cohort studies, which are currently lacking. We focused on the diagnostic significance of miRNA expression in different biological samples such as sera and tissues under the gold standard (pathological diagnosis) in patients with colorectal cancer. Although our study confirmed that miR-141 is a high-risk diagnostic marker for the early diagnosis of CRC, patients should be verified by pathological diagnosis.

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