Prognostic significance of microRNA-200c in various types of cancer: An updated meta-analysis of 34 studies

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Abstract. Previous studies have indicated that miR-200c is a promising cancer biomarker. However, different studies have presented conflicting results. Therefore, the aim of the present study was to perform a meta-analysis of miR-200c based on 34 relevant studies. The Materials and methods sections of papers were carefully identified using the databases PubMed, Web of Science and Embase for publications up to December 4, 2015. Pooled hazard ratios (HRs) and 95% confidence intervals (95% CIs) were systematically calculated to investigate the association between the expression of miR-200c and cancer prognosis. The results demonstrated that elevated expression levels of miR-200c indicated significantly worse overall survival rates (HR=1.37, 95% CI: 1.01, 1.85), and a high level of miR-200c was considered an indicator of an unfavorable prognosis in patients from Europe and America (HR=1.85, 95% CI: 1.27, 2.69). Furthermore, overexpression of miR-200c was significantly associated with progression of the disease in the subgroups of tissue and blood samples (HR=0.68 and 2.45, respectively), and inferior overall survival rates for the blood subgroup were revealed (HR=2.21, 95% CI: 1.04, 4.72). In addition, miR-200c was of prognostic value in several disease subgroups. Taken together, high expression levels of miR-200c are of significant prognostic value in various human malignancies.

Introduction

Cancer has become the primary cause of mortality in the majority of countries and regions worldwide, and the incidence of cancer has increased substantially in recent years (1). In 2012, 14.1 million new cancer cases, 8.2 million cancer mortalities and 32.6 million individuals living with cancer (within 5 years of diagnosis) were reported worldwide. Specifically, 57% (8 million) of new cancer cases, 65% (5.3 million) of cancer mortalities and 48% (15.6 million) of the 5-year prevalent cancer cases occurred in less developed regions (2). Due to the difficulty of early diagnosis and the low survival rate of multiple cancer types, reliable biomarkers that are associated with the diagnosis or prognosis of cancer are urgently required.

MicroRNAs are conserved small non-coding RNAs with a length of ~18-25 nucleotides, which regulate the expression of target genes and exert vital roles in various biological processes (3,4). They were first identified in 1993 (5). Thereafter, an understanding of their roles in the cell cycle, apoptosis, proliferation and differentiation has greatly advanced (6,7). Furthermore, several microRNAs were identified that function as either oncogenes or tumor suppressors, and the expression levels of certain microRNAs were associated with the degree of malignancy (8-10). Due to their good stability and unique expression profiles in human malignancies, microRNAs hold great promise as conceivable biomarkers for cancer diagnosis and prognosis (11,12).

MicroRNA-200c (miR-200c), the most representative member of the microRNA-200 family, has been widely investigated during the last few years. miR-200c was revealed to exert a critical role in the regulation of epithelial-to-mesenchymal transition (EMT) and mesenchymal-to-epithelial transition (MET) (13,14). In addition, there have been numerous studies demonstrating the association between an aberrant expression level of miR-200c and the prognosis of various human malignancies, including endometrial cancer (8,9), gastric cancer (13,15-17), ovarian cancer (18-21), clear cell renal cell carcinoma (ccRCC) (22-24), breast cancer (25-28), colorectal cancer (14,29), non-small cell lung cancer (NSCLC) (30-35), prostate cancer (36), esophageal cancer (37-39), diffuse large B-cell lymphoma (40), bladder cancer (41,42) and pancreatic cancer (43). Approximately half of these studies verified the anti-oncogenic function of miR-200c in certain cancer types, indicating the potential correlation of elevated expression levels of miR-200c and superior prognosis (13-15,18,20-22,28,29,31,35,41-43). However, other studies have provided opposing evidence, suggesting that miR-200c serves as an oncogene (23-27,36-39). Therefore, miR-200c is a noteworthy biomarker for cancer prognosis, and a meta-analysis of its precise role is required. To clarify

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the value of miR-200c as a prognostic biomarker, data from studies of miR-200c in various cancer types were systematically collected and evaluated.

Materials and methods

Search strategy. Relevant studies were identified by carefully searching the online databases PubMed, Web of Science and Embase up to December 4th, 2015. The following combination of keywords was simultaneously applied for the literature search: 'microRNA-200c' or 'microrna-200c' or 'miRNA-200c' or 'miR-200c' and 'tumor' or 'cancer' or 'carcinoma' or 'neoplasm' or 'malignancies'. In addition, the following criteria for the study characteristics were used to improve the search further: i) English language publications; ii) studies that concentrated on patients with malignancies; and iii) studies that demonstrated the association of miR-200c expression with cancer prognosis. This comprehensive online search was independently performed by two authors (Jia-Yi Zhang and Ya-Min Wang).

Inclusion and exclusion criteria. The present meta-analysis was performed strictly following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (44). Articles were considered eligible if they met the following criteria: i) the expression level of miR-200c had been assessed in tissue or peripheral blood samples from cancer patients; ii) dichotomous categorization of expression levels of miR-200c had been investigated according to a cut-off value; and iii) an investigation had been made of the association of expression levels of miR-200c with survival rates or recurrence, together with a corresponding hazard ratio (HR) or survival curve. If more than one article had been published on the identical study cohort, only the most comprehensive study was selected for the present meta-analysis. In addition, letters, review articles and experiments on animals were excluded. A flow diagram of the study selection process with further details is shown in Fig. 1.

Data extraction. All eligible studies were identified by Ya-Min Wang and Le-Bin Song, and uncertain data were reassessed by Ning-Hong Song. The data extraction included the following elements: i) the first author and publication year; ii) characteristics of the studied population, including patient nationality, number, mean or median age, disease type and stage, and sample examined; iii) study design, assay method and cut-off definition; iv) HRs of elevated expression levels of miR-200c for cancer-specific survival (CSS), overall survival (OS), recurrence-free survival (RFS), progression-free survival (PFS) and disease-free survival (DFS); and v) mean or median follow-up duration. If HRs were not directly reported in the studies, then the data were extracted from Kaplan-Meier survival plots using Engauge Digitizer v.5.1 (license type: GPL; developed by Mark Mitch; Category: C:\Science/CAD) to calculate HRs with 95% confidence intervals (95% CIs) using methods that are previously described (45). Furthermore, if both the univariate and multivariate results were reported, then only the latter was selected, since these results were adjusted for confounding factors. All the above-mentioned data are comprehensively shown in Tables I and II.

Statistical analysis. In the present meta-analysis, HRs and corresponding 95% CIs were combined to estimate the value of high expression levels of miR-200c for cancer prognosis. An individual or pooled HR of >1.0 indicated poorer prognosis in patients with miR-200c overexpression, and an HR of <1.0 represented an improved prognosis. Furthermore, a fixed-effects model using the Mantel-Haenszel method or a random-effects model using the DerSimonian-Laird method was applied for the meta-analysis, according to the heterogeneity between the pooled studies (46). Statistical heterogeneity was evaluated by performing the Chi-square test (assessing the P-value) and by calculating the Higgins I^2 statistic. If significant heterogeneity was observed ($P<0.10$ or $I^2>50\%$), the random-effects model was applied; otherwise, the fixed-effects model was used. Subgroup analyses were further performed to investigate the source of the identified heterogeneity. In addition, sensitivity analyses were implemented to avoid biases in the results due to certain low-quality studies, and the publication bias was estimated using Begg's and Egger's tests. All P-values were two-sided, and $P<0.05$ was considered to indicate a statistically significant value. All statistical analyses were conducted using Stata v.12.0 (StataCorp, College Station, Texas, USA), and Microsoft Excel (v.2010, Microsoft Corporation, Redmond, Washington, USA).

Results

Summary of the included studies. In total, 987 articles were initially collected from a primary retrieval using the databases PubMed, Web of Science and Embase. Of these articles, 33 articles that included 34 studies [the article of Marchini *et al* (20) included independent studies of two different cohorts, tissue collections A and B (20)] were ultimately considered eligible by screening the titles, abstracts and full texts (Fig. 1). Of these studies of the association between expression levels of miR-200c and the survival rate or disease recurrence in human malignancies, 27 were retrospective, and seven were prospective. In total, 3,940 patients from China, Germany, Finland, Japan, Spain, Australia, South Korea, Belgium, Sweden, Poland, USA, Italy or Denmark were included; these patients were diagnosed with a variety of cancer types, including endometrial cancer (8,9), gastric cancer (13,15-17), ovarian cancer (18-21), ccRCC (22-24), breast cancer (25-28), colorectal cancer (14,29), NSCLC (30-35), prostate cancer (36), esophageal cancer (37-39), diffuse large B-cell lymphoma (40), bladder cancer (41,42) and pancreatic cancer (43). Tissue samples were predominantly used to determine expression levels of miR-200c, although six studies detected expression levels of miR-200c in serum or plasma, and one study used tissue and blood samples (14). To assess miRNA-200c expression, quantitative real-time polymerase chain reaction (RT-qPCR) had predominantly been used in 32 studies, and *in situ* hybridization (ISH) was performed in three studies. The characteristics of primary studies are systematically summarized in Table I.

Association of CSS/OS with miR-200c overexpression. In total, 26 studies were included in the meta-analysis of the association between miR-200c overexpression and CSS/OS, and a random-effects model was applied due to the high

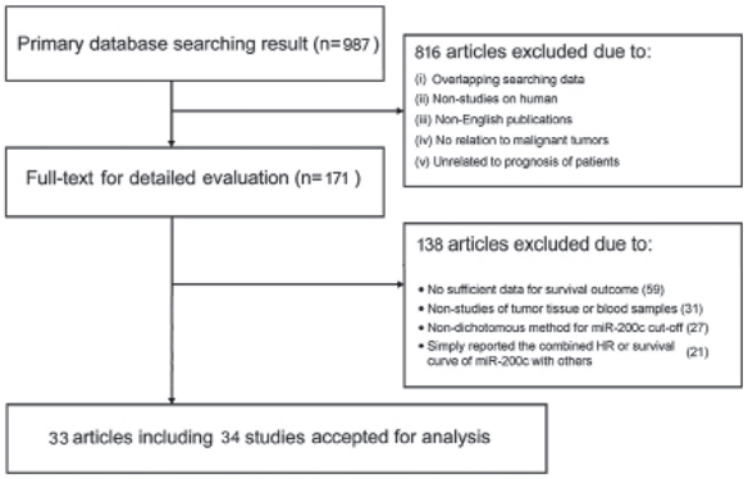

Figure 1. Flow diagram with details of the study selection process.

level of heterogeneity (P<0.001, I^2=86.2%). Three studies were excluded, since they enrolled cancer patients of only stage I (20,42). The pooled value of HRs from individual studies was 1.37 (95% CI: 1.01, 1.85), along with a P-value of 0.040 (Fig. 2A). Therefore, this result indicated a significant correlation of CSS/OS with high expression levels of miR-200c.

Furthermore, subgroup analyses were performed on specific study characteristics, including region, disease type and sample detection. All the pooled HRs with 95% CIs of the subgroups are shown in Fig. 2B and C. First, in the subgroup analysis of patients from Europe and America, the pooled outcome demonstrated that a high expression level of miR-200c was significantly associated with worse OS (HR=1.85, 95% CI: 1.27, 2.69). Secondly, the subgroups of esophageal cancer and endometrial cancer exhibited an identical association, with HR values of 1.68 and 2.18, respectively. However, the colorectal cancer subgroup demonstrated the opposite result (HR=0.54, 95% CI: 0.32, 0.90). Thirdly, the result for the blood sample subgroup significantly revealed that miR-200c overexpression was associated with worse OS, with an HR value of 2.21 (95% CI: 1.04-4.72). No significant results were identified in the other subgroups.

Association of disease progress with miR-200c overexpression. A total of 16 studies were included in the present meta-analysis of the association of miR-200c overexpression and RFS/PFS/DFS; the random-effects model was used due to the high level of heterogeneity (P<0.001, I^2=87.1%). Two studies from the literature were excluded, since they enrolled cancer patients at only stage I (20). The pooled HR from individual studies was 1.03, along with a P-value of 0.880, indicating a lack of statistical significance (Fig. 3A). Therefore, subgroup analyses were performed to reduce the confounding influence of the apparent heterogeneity (Fig. 3B).

First, high expression levels of miR-200c were shown to correlate significantly with improved disease progression in patients with NSCLC (HR=0.48, 95% CI: 0.34, 0.68; fixed-effects model: P=0.235, I^2=29.2% for the heterogeneity test). Secondly, the pooled HR of the tissue subgroup was 0.68 (95% CI: 0.48, 0.96, random-effects model; P<0.001, I^2=82.3% for the heterogeneity test), suggesting an association between miR-200c overexpression and favorable patient prognosis. However, the blood subgroup revealed a significant correlation of expression levels of miR-200c with unfavorable patient prognosis (HR=2.45, 95% CI: 1.85, 3.26; fixed-effects model: P=0.722, I^2=0.0% for heterogeneity test).

Sensitivity analysis. In the studies of CSS/OS and RFS/PFS/DFS, our sensitivity analyses did not reveal any alterations in the results due to the inclusion of any individual study (Fig. 4A and B), indicating that no single study significantly influenced the pooled HRs and 95% CIs.

Publication bias. Egger's test and Begg's funnel plot were used for the analysis of publication bias. The funnel plots of the CSS/OS and RFS/PFS/DFS analyses were almost symmetrical, and all P-values from the Egger's test were >0.05 (Fig. 4C and D). Therefore, no significant publication bias was observed in the present meta-analysis.

Discussion

The EMT is a well-established mechanism that includes intercellular contact disruption and enhanced cell motility (47). Additionally, a burgeoning body of evidence has clearly demonstrated the involvement of EMT in the invasion and migration of tumor cells (14,32,43). Intracellular and extracellular factors are known to be capable of promoting or inhibiting EMT progression. In particular, the miR-200 family has been proposed to suppress EMT by directly targeting the transcriptional repressors of E-cadherin, zinc finger E-box binding homeobox 1 (ZEB1) and zinc finger E-box binding homeobox 2 (ZEB2), thus inducing E-cadherin upregulation (48). Conversely, inhibition of the miR-200 family would induce mesenchymal-like spindle morphology, which promotes cancer metastasis.

As the most representative microRNA among the miR-200 family, miR-200c fulfills important roles in EMT inhibition and in MET promotion. For instance, Marchini *et al* (20) demonstrated that several downstream targets of miR-200c, including vascular endothelial growth factor A (VEGFA)

Table I. The predominant characteristics of the included studies.

Authors, year	Patient's nationality	Patient's number	Mean or median age	Study design	Malignant disease	Disease stage	Detected sample	Mean or median follow-up time	Refs.
Antolín *et al*, 2015	Spain	57	55.4	R	Breast cancer	I-IV	Blood	$264.6^{OS}/235.3^{PFS}$ weeks	(27)
Butz *et al*, 2015	Canada	425	NM	R	ccRCC	I-IV	Tissue	NM	(24)
Song *et al*, 2015	China	134	50.0	R	Breast cancer	I-IV	Tissue	NM	(28)
Zhao *et al*, 2015	China	78	61.4	P	NSCLC	IIB-IIIB	Tissue	NM	(35)
Zhou *et al*, 2015	China	63	65.0	R	Gastric cancer	IIB-IV	Tissue	NM	(13)
Gao *et al*, 2015	China	93	NM	R	Ovarian cancer	I-IV	Blood	NM	(18)
Vergho *et al*, 2014	Germany	37	66.8	R	ccRCC	I-IV	Tissue	45.6 months	(22)
Tuomarila *et al*, 2014	Finland	172	60.4	P	Breast cancer	I-IV	Tissue	9.7 years	(25)
Toiyama *et al*, 2014	Japan	$156^{T}/182^{S}$	68.0	R	Colorectal cancer	I-IV	Both	20 months	(14)
Tejero *et al*, 2014	Spain	155	65.0	R	NSCLC	I-III	Tissue	43 months	(30)
Song *et al*, 2014	China	373	60.5	R	Gastric cancer	I-IV	Tissue	35 months	(17)
Lin *et al*, 2014	Australia	97	68.0	P	Prostate cancer	III-IV	Blood	12 months	(36)
Li *et al*, 2014	China	150	59.0	R	NSCLC	IIIB, IV	Tissue	16.7 months	(31)
Kim *et al*, 2014	South Korea	72	64.0	R	NSCLC	I-IV	Tissue	31 months	(32)
Diaz *et al*, 2014	Spain	127	67.4	R	Colorectal cancer	I-III	Tissue	113 months	(29)
Cao *et al*, 2014	China	100	58.0	R	Ovarian cancer	I-IV	Tissue	36.8 months	(19)
Berghmans *et al*, 2013	Belgium	38	59.0	P	NSCLC	NM	Tissue	NM	(33)
Yu *et al*, 2013	China	157	60.0	R	Esophageal cancer	III, IV	Blood	20 months	(37)
Wotschofsky *et al*, 2013	Germany	111	>60.0	R	ccRCC	NM	Tissue	NM	(23)
Tang *et al*, 2013	China	167	60.0	R	Gastric cancer	I-IV	Tissue	NM	(15)
Tanaka *et al*, 2013	Japan	64	67.5	R	Esophageal cancer	II-IV	Blood	NM	(38)
Berglund *et al*, 2013	Sweden	61	>60.0	R	DLBCL	I-IV	Tissue	NM	(40)
Madhavan *et al*, 2012	Germany	193	NM	R	Breast cancer	III-IV	Blood	NM	(26)
Valladares-Ayerbes *et al*, 2012	Spain	52	65.3	P	Gastric cancer	I-IV	Blood	24 months	(16)
Torres *et al*, 2013	Poland	108	62.8	P	Endometrial cancer	I-IV	Tissue	NM	(9)
Liu *et al*, 2012	China	70	60.0	P	NSCLC	I-IV	Tissue	NM	(34)
Karaayvaz *et al*, 2012	USA	34	NM	R	Endometrial cancer	I-IV	Tissue	NM	(8)
Wszolek *et al*, 2011	USA	57	>60.0	R	Bladder cancer	NM	Tissue	92 months	(41)
Marchini *et al*, 2011	Italy	89	52.0	R	Ovarian cancer	I	Tissue	110 months	(20)
Marchini *et al*, 2011	Italy	55	57.0	R	Ovarian cancer	I	Tissue	108 months	(20)
Hamano *et al*, 2011	Japan	98	>60.0	R	Esophageal cancer	I-IV	Tissue	28.8 months	(39)
Wiklund *et al*, 2011	Denmark	100	NM	R	Bladder cancer	I	Tissue	NM	(42)
Yu *et al*, 2010	Japan	99	65.7	R	Pancreatic cancer	I-IV	Tissue	NM	(43)
Leskelä *et al*, 2010	Spain	72	57.0	R	Ovarian cancer	I-IV	Tissue	NM	(21)

The study design is described as prospective (P) or retrospective (R) T, tissue sample of patients was assayed; S, serum sample of patients was assayed; ccRCC, clear cell renal cell carcinoma; NSCLC, non-small cell lung cancer; DLBCL, diffuse large B-cell lymphoma; NM, not mentioned; OS, overall survival; PFS, progression-free survival.

Table II. HRs of included studies.

Authors, year	Main assay of miR200c	Cut-off	Resource of HR	HRs		Refs.
				OS/CSS	RFS/PFS/DFS	
Antolín *et al*, 2015	RT-qPCR	Mean	Reported	2.79	3.33	(27)
Butz *et al*, 2015	RT-qPCR	Mean	Reported	2.73	3.57	(24)
Song *et al*, 2015	ISH	Mean	[a]	0.18	0.19	(28)
Zhao *et al*, 2015	RT-qPCR	Median	Reported	-	0.35	(35)
Zhou *et al*, 2015	RT-qPCR	Median	[a]	-	0.49	(13)
Gao *et al*, 2015	RT-qPCR	Mean	Reported	0.32	-	(18)
Vergho *et al*, 2014	RT-qPCR	2.73	Reported	0.95	-	(22)
Tuomarila *et al*, 2014	RT-qPCR	Median	[a]	2.78	3.16	(25)
Toiyama *et al*, 2014	RT-qPCR	Median	Reported	$0.56^{T}/2.67^{S}$	4.51^{S}	(14)
Tejero *et al*, 2014	RT-qPCR	Mean	[a]	1.95	-	(30)
Song *et al*, 2014	RT-qPCR	Lowest quartile	Reported	1.32	1.06	(17)
Lin *et al*, 2014	RT-qPCR	Median	Reported	2.30	-	(36)
Li *et al*, 2014	RT-qPCR	0.01385	Reported	0.57	0.55	(31)
Kim *et al*, 2014	RT-qPCR	Mean	Reported	3.67	-	(32)
Diaz *et al*, 2014	RT-qPCR	NM	[a]	0.51	0.55	(29)
Cao *et al*, 2014	RT-qPCR	3.84	Reported	16.22	-	(19)
Berghmans *et al*, 2013	RT-qPCR	Median	Reported	1.51	-	(33)
Yu *et al*, 2013	RT-qPCR	Median	Reported	1.67	-	(37)
Wotschofsky *et al*, 2013	RT-qPCR	Median	Reported	-	1.40	(23)
Tang *et al*, 2013	RT-qPCR/ISH	Mean	Reported	0.40	0.51	(15)
Tanaka *et al*, 2013	RT-qPCR	Median	Reported	-	2.79	(38)
Berglund *et al*, 2013	RT-qPCR	Mean	[a]	2.68	-	(40)
Madhavan *et al*, 2012	RT-qPCR	Lower quartile	[a]	15.27	2.20	(26)
Valladares-Ayerbes *et al*, 2012	RT-qPCR	62.4	Reported	2.24	2.27	(16)
Torres *et al*, 2013	RT-qPCR	Median	Reported	2.72	-	(9)
Liu *et al*, 2012	RT-qPCR	2.00	Reported	6.02	-	(34)
Karaayvaz *et al*, 2012	RT-qPCR	35.5	[a]	1.28	-	(8)
Wszolek *et al*, 2011	RT-qPCR	Mean	[a]	0.09	-	(41)
Marchini *et al*, 2011	RT-qPCR	Median	Reported	0.24	0.42	(20)
Marchini *et al*, 2011	RT-qPCR	Median	Reported	0.09	0.04	(20)
Hamano *et al*, 2011	RT-qPCR	Median	[a]	1.71	-	(39)
Wiklund *et al*, 2011	ISH	NM	[a]	0.52	-	(42)
Yu *et al*, 2010	RT-qPCR	0.64	Reported	0.45	-	(43)
Leskelä *et al*, 2010	RT-qPCR	Median	Reported	-	0.85	(21)

The study design is described as prospective (P) or retrospective (R). [a]Data extracted from the survival curve. In the OS/CSS and RFS/PFS/DFS columns, 'T' denotes the HR of miR-200c overexpression in tumor tissue, and 'S' denotes the HR of miR-200c overexpression in serum sample; HR, hazard ratio; OS, overall survival; CSS, cancer specific survival; RFS, relapse-free survival; PFS, progression-free survival; DFS, disease-free survival; RT-qPCR, quantitative reverse transcription-polymerase chain reaction; ISH, *in situ* hybridization.

and tubulin, beta 3 class III (TUBB3), were significantly upregulated in patients with ovarian cancer who relapsed (20). Leskelä *et al* (21) suggested an inverse correlation between miR-200c and TUBB3 expression in advanced ovarian cancer. Furthermore, a marked inverse correlation of the expression levels of miR-200c and the mRNA levels of VEGFA was demonstrated in two independent cohorts of ccRCC and normal tissues (49). Thus, miR-200c has been considered a tumor suppressor.

However, a potential oncogenic role of miR-200c in human malignancies has also been reported. Tuomarila *et al* (25) demonstrated that progesterone receptor (PR)-negative cases with local or distant recurrence had higher expression levels of miR-200c compared with those without recurrence, suggesting that a high expression level of miR-200c is an independent factor for predicting poor survival rates in PR-negative breast cancer. Tejero *et al* (30) reported that high expression levels of miR-200c were associated with shorter OS of patients with NSCLC due to MET and angiogenesis (30). In addition, Hamano *et al* (39) indicated that the miR-200c-induced chemoresistance of esophageal cancer was mediated by the Akt pathway, showing that miR-200c overexpression was significantly correlated with a shortened OS (39).

In the present meta-analysis, a significant association of the expression of miR-200c with outcome was observed for pooled CSS/OS (Fig. 2A). However, there was heterogeneity

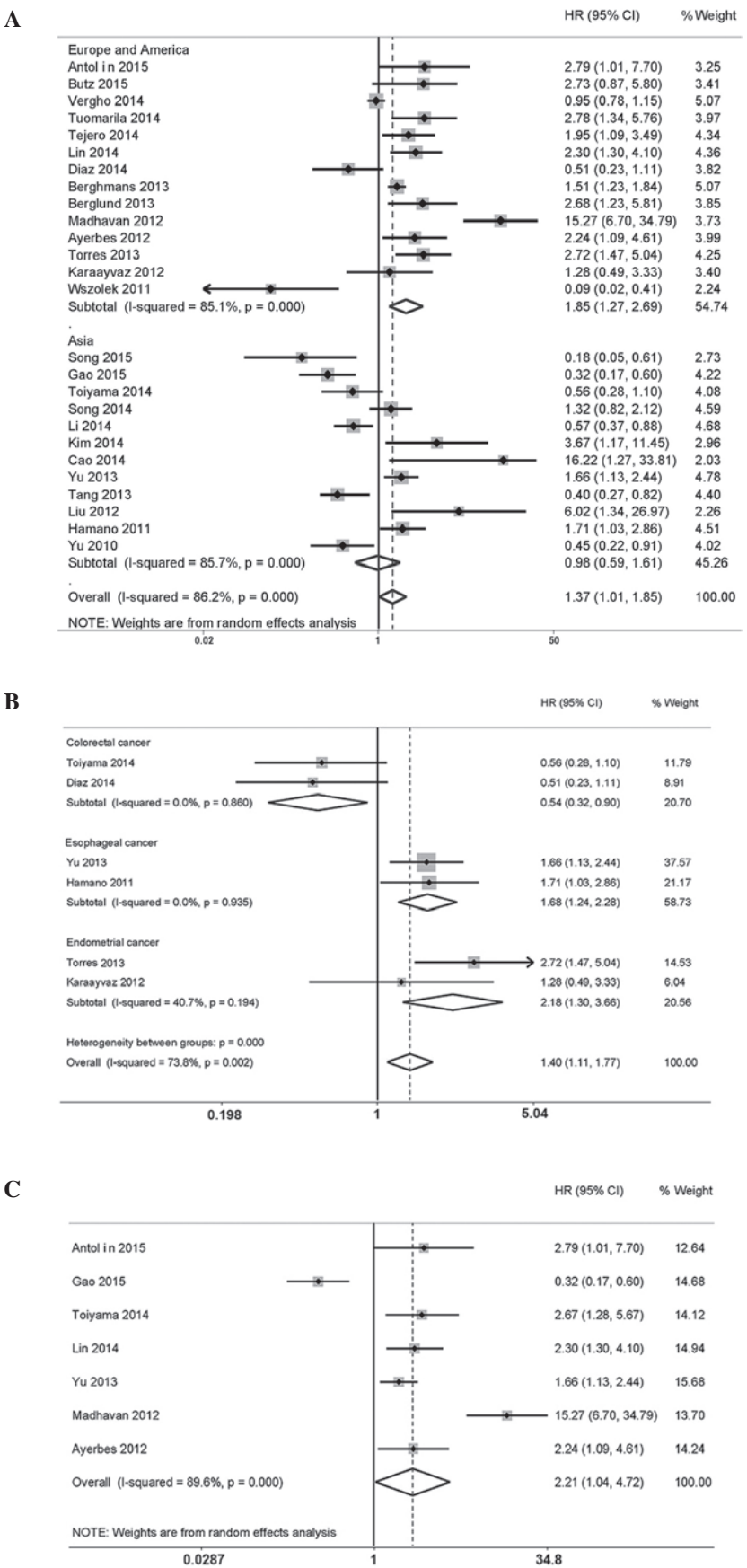

Figure 2. Forest plots of the combined analyses of the association of CSS/OS and expression levels of miR-200c. (A) Forest plots of the pooled analysis of CSS/OS. Squares and horizontal lines correspond to study-specific HRs and 95% CIs, respectively. The area of the squares correlates with the weight, and the diamonds represent the pooled HRs and 95% CIs. (B) Forest plots of the pooled analysis of CSS/OS in different disease type subgroups. (C) Forest plots of the pooled analysis of CSS/OS in blood sample subgroup. CSS, cancer-specific survival; OS, overall survival; HR, hazard ratio; CI, confidence interval.

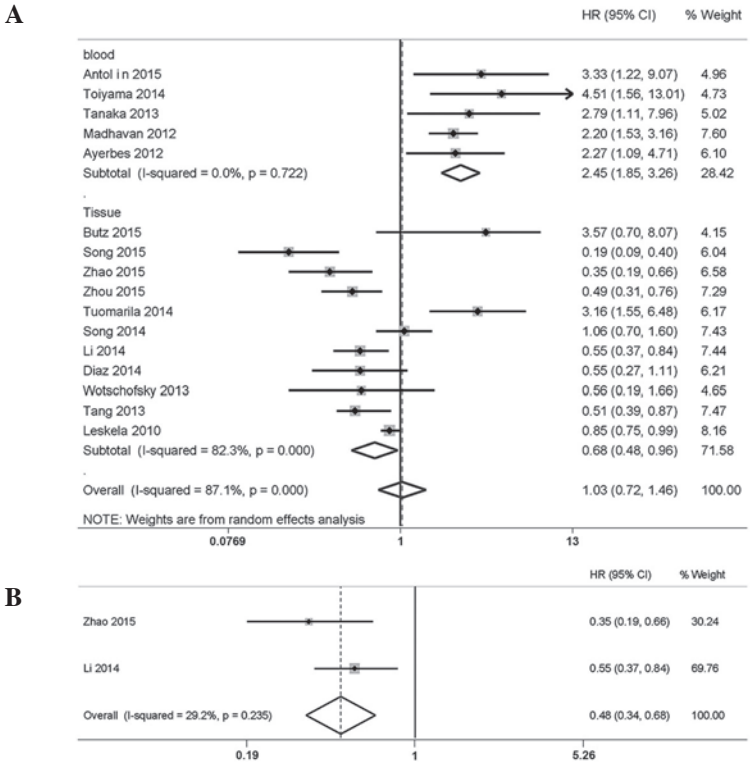

Figure 3. Forest plots of the pooled analysis of (A) the association of RFS/PFS/DFS and miR-200c expression in different sample subgroups, and (B) RFS/PFS/DFS in the in the non-small cell lung cancer subgroup. RFS, recurrence-free survival; PFS, progression-free survival; DFS, disease-free survival; HR, hazard ratio; CI, confidence interval.

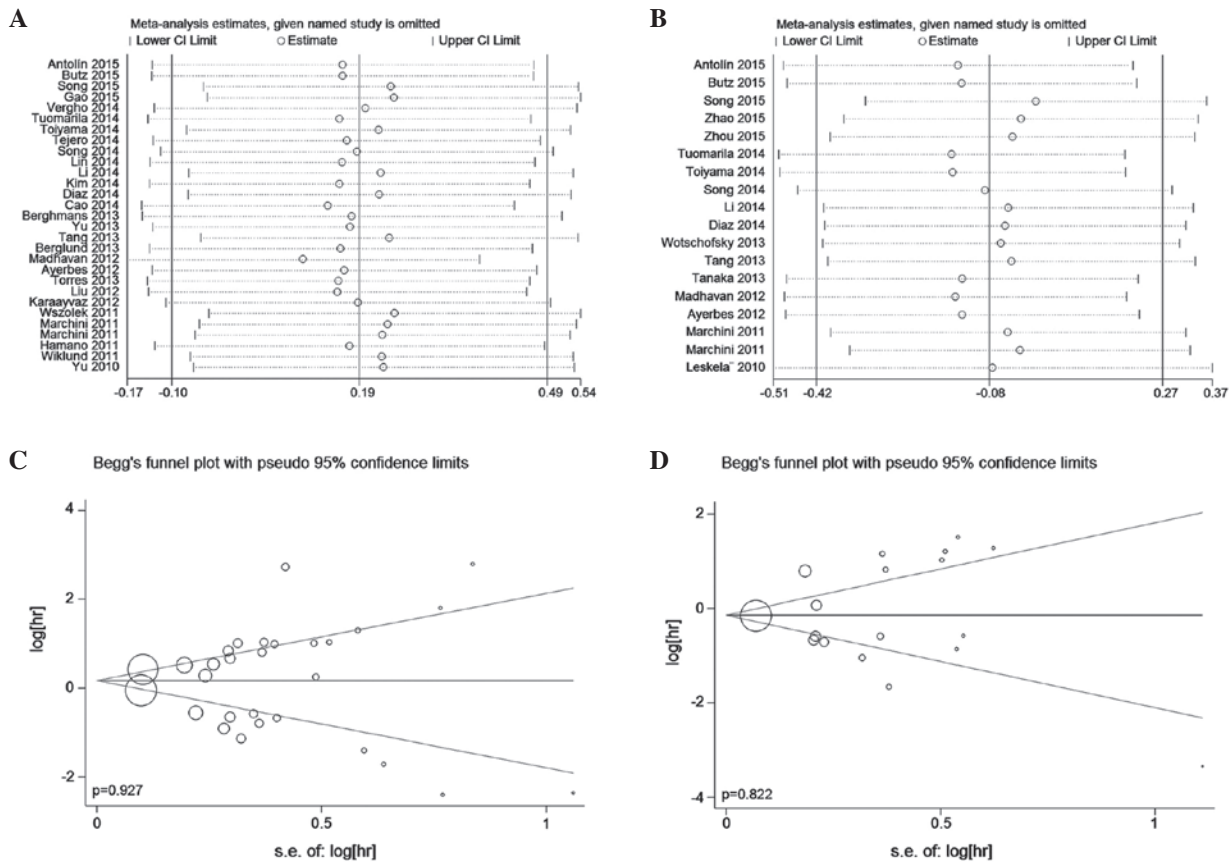

Figure 4. Sensitivity analyses and Begg's funnel plots. (A) Sensitivity analysis of the effect of individual studies on the CSS/OS results. (B) Sensitivity analysis of the effect of individual studies on the RFS/PFS/DFS results. (C) Begg's funnel plots to test for the publication bias in the overall analysis of CSS/OS. Each point represents a separate study. (D) Begg's funnel plots to test for publication bias in the overall analysis of RFS/PFS/DFS. RFS, recurrence-free survival; PFS, progression-free survival; DFS, disease-free survival; HR, hazard ratio; CI, confidence interval.

in both groups of outcomes (P<0.001, I^2=86.2% for CSS/OS; P<0.001, I^2=87.1% for RFS/PFS/DFS). Heterogeneity may be caused by the different characteristics of the patients, including race, disease type and clinical stage, as well as the selected cut-off value of the expression level of miR-200c. To minimize the influence of these confounding factors, subgroup analyses that focused on region, disease type and sample detection were performed. On the basis of the subgroup analyses of the included studies, heterogeneity in several subgroups was greatly reduced, and valuable results were obtained (Fig. 2B and C). These results indicate that miR-200c may be used as a prognostic biomarker; however, several details require further refinement. First, different cut-off values were used in the studies of miR-200c. The majority of the available studies established a median or mean expression cut-off value, although certain studies used a lower quartile value. Furthermore, several studies used a ternary method, separating the expression levels of miR-200c into high, intermediate and low categories (50). Due to the lack of standardized miR-200c expression data, evaluating its prognostic role in malignancies would produce inaccurate results. Secondly, whether miR-200c functions as an independent tumor biomarker or, more likely, as a component of a predictive microRNA signature, has yet to be firmly established. For instance, Yeh *et al* (51) demonstrated that the downregulation of miR-141 and miR-200c serves as an independent predictor of DFS in hepatocellular carcinoma. In the multivariate analysis performed by Blanco-Calvo *et al* (52), the combination of high levels of growth differentiation factor 15, matrix metalloproteinase 7 and miR-200c was considered an independent predictor of mortality in gastric cancer. In addition, using a linear combination of the microRNA cycle threshold values and Cox regression coefficients as weights, Berghmans *et al* (33) revealed that a certain microRNA signature (miR-200c, miR-124, miR-29c and miR-424) is of prognostic value for OS in patients with NSCLC (33). Thirdly, although significant results were identified for several subgroups based on the subgroup analyses, the results for other subgroups were not decisive. Empirically, a prognostic factor is considered decisive when the HR is >2.0 or < 0.5 (53). Finally, several HRs were extracted from the survival curves, which unavoidably resulted in several slight statistical errors.

In conclusion, we have demonstrated that high expression levels of miR-200c are of significant prognostic value in various human malignancies. In addition, expression levels of miR-200c in tumor tissue and blood samples were considered to serve as a reliable predictive biomarker for disease progression in cancer patients. Due to the complex role of miR-200c in tumor progression and metastasis, further investigations at a larger scale are required to establish the usefulness of miR-200c as a prognostic biomarker.

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