Original Article
Prognostic and clinicopathological significance of microRNA-200 family in lung cancer: a meta-analysis

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Abstract: *Background/Aim:* In recent years, the relationship between miR-200 expression and prognosis in lung cancer patients has been studied extensively. The miR-200 family plays a vital role in occurrence, development, metastasis, and drug resistance of lung cancer. Increasing evidence has shown that the miR-200 family is highly expressed in lung cancer and associated with poor prognosis. However, results have been inconclusive. Therefore, the aim of this study was to assess the association between miR-200 family expression and the clinicopathological and prognostic significance in patients with lung cancer via conducting a meta-analysis. *Methods:* A total of 9 different publications were searched from PubMed, Web of Science, Embase, and Cochrane library databases. Summary hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated using random-effects/fixed-effects models. *Results:* A total of nine articles, consisting of 812 patients, were included in this meta-analysis. Pooling all eligible studies, this study found a shorter overall survival in patients with high expression of the miR-200 family (HR = 2.29, 95% CI: 1.10-4.73). Furthermore, subgroup analysis, based on area, demonstrated that a significant association was found between higher expression of the miR-200 family and poor overall survival (HR = 4.70, 95% CI: 1.59-13.89) in the European population. However, there was no significant association between high expression of the miR-200 family and poor PFS (HR = 1.40, 95% CI: 0.21-9.25). In addition, this study failed to observe a correlation of increased miR-200 family expression with clinicopathological parameters, including age, sex, histology, tumor node metastasis (TNM) stage, lymph node metastasis, and tumor differentiation. *Conclusion:* High expression of the miR-200 family is significantly associated with poor clinical outcomes, particularly decreasing overall survival for European populations. Future studies should be performed to confirm the clinical utility of the miR-200 family in lung cancer.

Keywords: miR-200, lung cancer, prognosis, meta-analysis

Introduction

MicroRNAs (miRs/miRNAs) are characterized by a class of small 19-22 nucleotides in length, non-coding, endogenous, single-stranded, and highly conserved RNAs. They have been associated with post-transcriptional gene regulation. They bind to the complementary sequences of 3'-untranslated regions (3'-UTR) of target genes, negatively regulating expression of numerous genes and suppressing translation to proteins [1]. Consequently, this facilitates translational silencing or mRNA degradation of targeted genes. Relative studies had proven that miRNAs could play either tumor-suppres-

sive or oncogenic roles in a variety of pathways according to the cellular context or the target genes. For example, relative studies [2, 3] have reported that miRNAs are involved in various cancers, such as lung cancer, ovarian cancer, breast cancer, colorectal cancer, liver cancer, and so forth, suggesting that they play a critical role in cancer. miR-200, a family of tumor suppressor miRNAs, consists of five members, miR-200a, miR-200b, miR-429 comprise cluster 1, which is located on chromosome 1p36, miR-200c, and miR-141 comprise cluster 2, which is located on chromosome 12p13 [4]. Many studies [5-7] have shown that the miR-200 family is involved in the inhibition of epithe-

lial-to-mesenchymal transition (EMT), repression of cancer stem cell self-renewal and differentiation, modulation of cell division and apoptosis, and reversal of chemoresistance.

The leading cause of cancer-associated morbidity and mortality, both in men and women worldwide, is lung cancer, with approximately 222,500 new cases of lung tumors in the United States [8]. Between 85% of lung cancer cases are diagnosed as non-small cell lung cancer (NSCLC), which has a very low overall 5-year survival rate (about 10%) [9]. The remaining cases are small-cell lung cancer (SCC), which is more aggressive but occupies just 10-15% [10, 11].

Recent investigations have shown that expression levels of the miR-200 family are associated with prognostic and clinical outcomes in NSCLC and miR-200 family expression has value as an independent prognosis factor. However, the precise roles of the miR-200 family in lung cancer remain controversial. In 2010, Paolo Ceppi et al. [12] found that patients with lower miR-200c expression levels had poorer prognosis. They were linked to a higher propensity to lymph node metastases, poor grade of differentiation, and lower survival. Li et al. [13] holds the view that high a level of miR-200c expression is associated with longer overall survival. In contrast, Si et al. [14] demonstrated that overexpression miR-200c is significantly associated with poor survival and positive lymph node metastasis. The current meta-analysis investigated the roles of the miR-200 family in lung cancer.

Material and methods

Literature search

To search potentially eligible studies, a comprehensive literature retrieval was conducted using PubMed, Embase, Cochrane library databases, and Web of Science, with a cut-off date of October 30th, 2018. Keywords included "miR-200", "miR-200a", "miR-200b", "miR-200c", "miR-141", "miR-429", "lung", "cancer", "carcinoma", and "tumor". In addition, this study screened other relevant articles from the reference lists.

Inclusion and exclusion criteria

Eligible articles had to meet the following criteria: (1) Investigation of the roles of the miR-200 family in development of human cancer; (2) Description of the association of miR-200 family expression with prognosis or clinicopathological features; (3) Patients were divided into high and low expression groups based on expression levels of the miR-200 family; (4) Sufficient data for examining hazard ratios (HRs) with 95% confidence intervals (CIs) for overall survival (OS) or progression-free survival (PFS); and (5) No patients with HIV or acute disease. Exclusion criteria were as follows: (1) Duplications of the previous publications; (2) Studies without valuable data; and (3) Case reports, reviews, letters, and expert opinions.
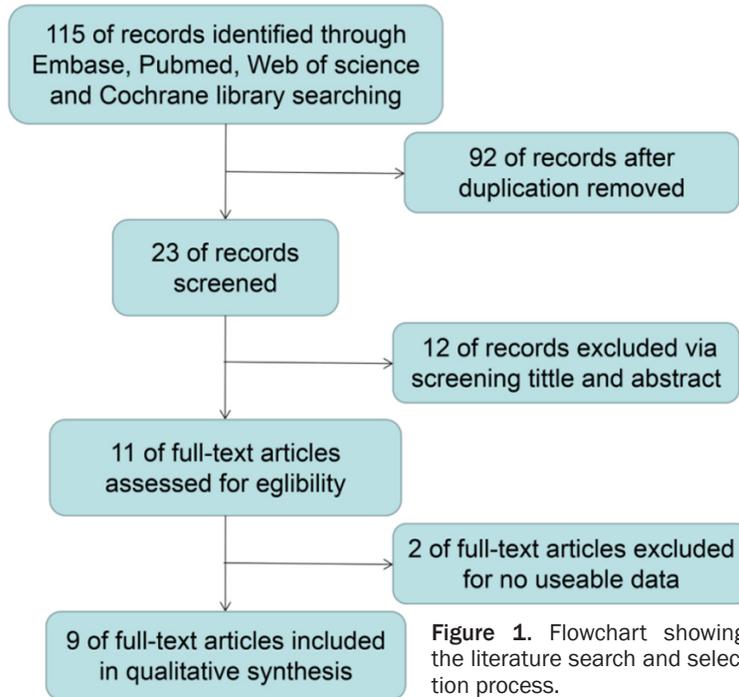
Date extraction and quality assessment

Data from all eligible studies were independently extracted by two reviews (PX and SYX). Detailed information was collected from each study, including first author, publication year, study country, total number of patients, follow-up period, tumor stage, types of miRNA, HRs, and corresponding 95% CIs of OS and PFS. Moreover, data concerning clinicopathological parameters were also extracted from studies. For included studies, only selected multivariate analyses were selected, due to the increased precision in interpreting confounding factors. Any studies representing only Kaplan-Meier curves, without the data above, were excluded. If there were disagreements, a consensus was settled by a third reviewer (BW). The Newcastle-Ottawa Scale (NOS) [15] was used to measure the quality of included studies. Assessment content included selection, exposure, and comparability. NOS scores ranged from 0 to 9. NOS scores ≥ 6 indicate high quality. The quality of all studies in this meta-analysis varied from 5 to 9, with a mean value of 7.0.

Statistical analysis

Pooled HRs and 95% CIs for clinical outcome endpoints (OS and PFS) from each eligible article were calculated to assess the relevance between miR-200 family expression and prognostic significance in patients with lung cancer. ORs and 95% CIs were calculated to evaluate the strength of association between miR-200 family expression and clinicopathological parameters (age, sex, histology, TNM stage, lymph node metastasis, and tumor differentiation). The current meta-analysis was conducted with Stata SE12.0 software and RevMan5.3. Heterogeneity assumption between

| 115 of records identified through Embase, Pubmed, Web of science and Cochrane library searching |
| 92 of records after duplication removed |
| 23 of records screened |
| 12 of records excluded via screening tittle and abstract |
| 11 of full-text articles assessed for eglibility |
| 2 of full-text articles excluded for no useable data |
| 9 of full-text articles included in qualitative synthesis |

Figure 1. Flowchart showing the literature search and selection process.

studies was assessed with the Chi-squared-based Q-test and I^2 statistic. *P* values of more than 0.10 for the Q test and an I^2 values smaller than 50% indicate no significant heterogeneity. A fixed-effects model (Mantel-Haenszel method) was applied for studies with no obvious heterogeneity (Ph>0.05, I^2<50%). Otherwise, a random-effects model (DerSimonian and Laird method) was chosen (Ph ≤ 0.05, I^2 ≥ 50%). Potential publication bias was assessed using both Begg's test and Egger's test [16]. Otherwise, sensitivity analysis was performed to determine the stability and effects of a single study on pooled HRs by deleting one study. *P* values less than 0.10 indicate statistical significance.

Results

Study characteristics

Figure 1 shows details of the literature retrieval process. A total of 115 articles were initially retrieved. Trimming unrelated studies, duplicate articles and letters, and screening full-texts, depending upon the search strategy, 9 eligible articles were ultimately identified. These articles included 812 lung cancer patients. The 9 included studies were from Italy, Spain, France, Korea, and China. Detailed characteris-

tics are shown in **Table 1**. All cancerous specimens were preserved before experiments were carried out.

miR-200 family overexpression and overall survival

Of the 9 eligible studies [12-14, 17-22], 8 studies reported the OS according to miR-200 family expression. A random-effects model was performed to estimate pooled HRs and corresponding 95% CIs. Results show that HR in the high miR-200 family expression group versus the low group was 2.29 (95% CI: 1.10-4.73), indicating high heterogeneity across the studies (I^2 = 85.2%, Ph<0.01) (**Figure 2A**). After stratification by area, HRs for the high miR-200 family expression group versus the low group were 1.61 (95% CI: 0.72-3.62) in Asia and 4.70 (95% CI: 1.59-13.89) in Europe (**Figure 2B**). After stratification by miR-200 family member types, HRs for the high miR-200 family expression group versus the low group were 2.10 (95% CI: 0.86-5.12) for miR-200c, 2.75 (95% CI: 0.71-10.71) for miR-429, and 2.97 (95% CI: 1.25-7.04) for miR-141 (**Figure 2C**). Overall, results indicate a significant difference in OS between the two groups. In addition, stratified analysis by region revealed significant association between high miR-200 family expression and poor OS in European populations, compared with Asian populations. Thus, it was concluded that higher expression of the miR-200 family was associated with poor OS, especially for European populations.

miR-200 family overexpression and progression-free survival

Only 2 studies, including a total of 188 patients, provided appropriate data for analysis of PFS. A random effects model was applied to analyze pooled HRs with their corresponding 95% CIs due to severe statistical heterogeneity (I^2 = 94.1%; Ph<0.01). However, there was no significant association between high expression of the miR-200 family and poor PFS (HR = 1.40, 95% CI: 0.21-9.25) (**Figure 3**).

Table 1. Main characteristics of all included studies

Author	Publication year	Country	miRNA	Total number	TNM stage	Following year (months)	Detection method	Outcome measure	Multivariate analysis	Type of data
Si	2017	China	miR-200c	110	91/19 I-II/III	72	RT-qPCR	OS DFS	Yes	High vs. Low
Zhao	2015	China	miR-200c	78	21/57 II/III-IV	40	RT-qPCR	OS	Yes	High vs. Low
Kim	2014	Korea	miR-200c	72	37/35 I-II/III-IV	125	RT-qPCR	OS	Yes	High vs. Low
Li	2014	China	miR-200c	150	20/130 III/IV	40	RT-qPCR	OS PFS	Yes	High vs. Low
Zhu	2014	China	Mir-429	70	36/34 I/II-IV	30	RT-qPCR	OS	Yes	High vs. Low
Berghmans	2013	Franc	miR-200c	38	NR	60	Biopsy	OS PFS	Yes	High vs. Low
Liu	2012	China	miR-200c	70	36/34 I/II-IV	30	RT-qPCR	OS	Yes	High vs. Low
Liu	2012	China	Mir-141	70	36/34 I/II-IV	30	RT-qPCR	OS	Yes	High vs. Low
Tejero	2014	Spain	miR-200c	155	128/27 I-II/III	150	RT-qPCR	OS	Yes	High vs. Low
Tejero	2014	Spain	Mir-141	155	128/27 I-II/III	150	RT-qPCR	OS	Yes	High vs. Low
Paolo	2010	Italy	miR-200c	69	55/14 I-II/III	NR	RT-qPCR	NR	Yes	High vs. Low

Abbreviations: TNM, tumor node metastasis; DFS, disease-free survival; PFS, progression-free survival; OS, overall survival; NR, not reported.

Association between miR-200 family overexpression and clinicopathological parameters

Results of pooled analysis are presented in **Table 2**. Unfortunately, no significant correlation was observed between overexpression of the miR-200 family and age, sex, histology, TNM stage, lymph node metastasis, and tumor differentiation (forest plot not shown). Due to insufficient data, this study failed to find a relationship between miR-200 family overexpression and other clinicopathological parameters.

Sensitivity analysis

To analyze the association between overexpression of miR-200 family levels and OS, sensitivity analysis was conducted by removing each study, in turn, from pooled analysis. The aim of this process was to assess the influence of the removed data set on overall HRs. Results were not significantly affected by the exclusion of any article, indicating the robustness of present results.

Publication bias

To analyze the association between overexpression of miR-200 family levels and OS, Egger's test and Begg's test were adopted to test publication bias. Results showed no publication bias among included studies (**Figure 4**).
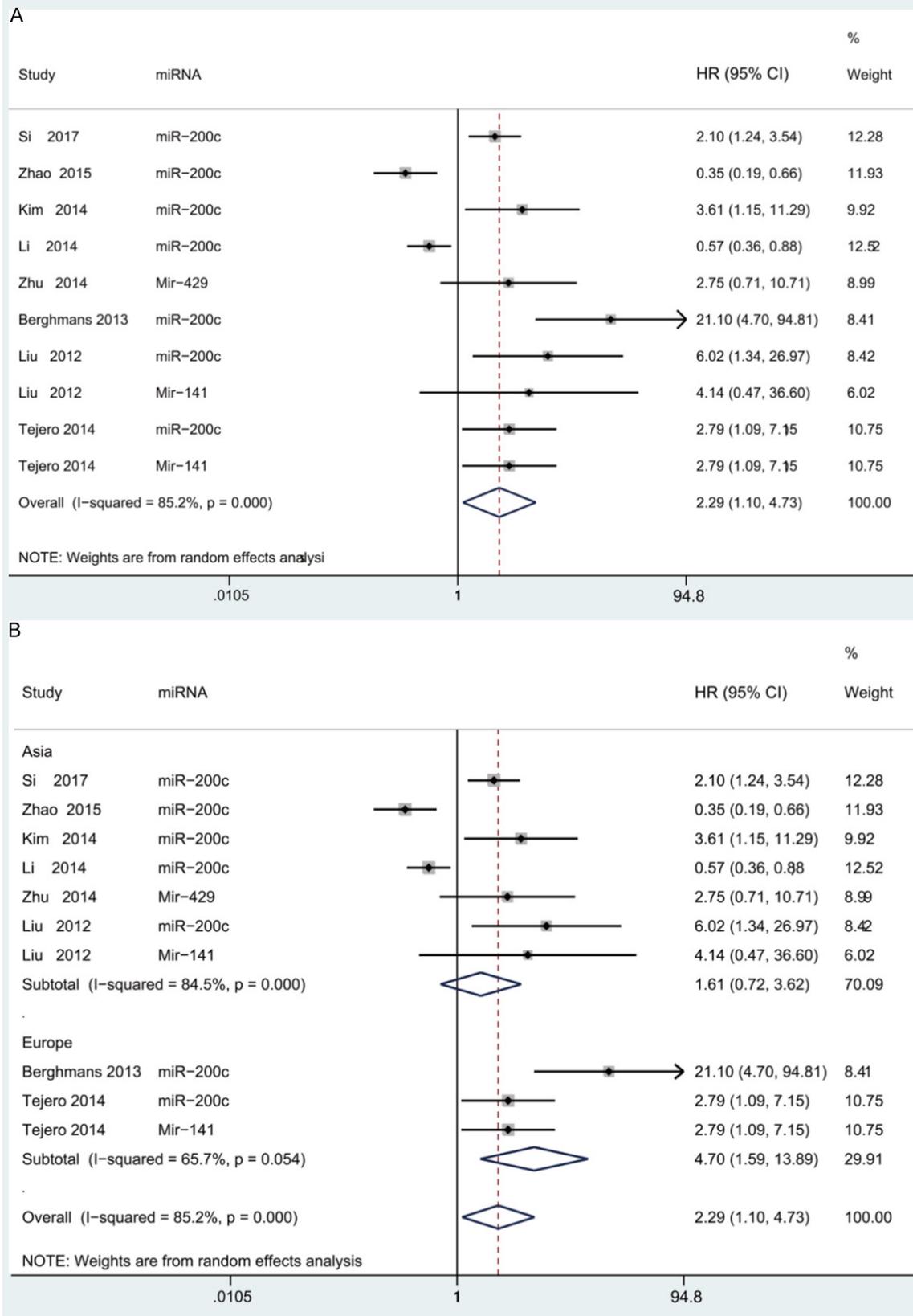
Discussion

The current study evaluated the association between miR-200 family expression and clinical outcomes of lung cancer. Pooled analysis of nine studies (with 812 patients) demonstrated

that higher expression of the miR-200 family significantly cut down OS. Results of this meta-analysis show a shorter OS in patients with higher expression of the miR-200 family (HR = 2.29, 95% CI: 1.10-4.73). However, no significant association was detected between poor PFS with levels of the miR-200 family (HR = 1.40, 95% CI: 0.21-9.25), implying that expression of the miR-200 family was a hazardous factor for clinical outcomes of lung cancer patients. In addition, this study checked the link between higher miR-200 levels and pathological features (**Table 2**). Unfortunately, there was no significant correlation between the miR-200 family and age, sex, histology, TNM stage, lymph node metastasis, and tumor differentiation.

The miR-200 family has been associated with restriction of EMT and metastasis in cancer cell lines derived from murine that develop metastatic lung adenocarcinoma and primary lung cancers from multiple TCGA dataset having high EMT scores [23, 24]. EMT involves profound phenotypic changes that contain the loss of cancer cell's epithelial properties, such as cell-cell adhesion or cell polarity and the acquisition of a migratory and invasive phenotype that facilitates metastasis. Specific micro-ribonucleic acids have also been found to regulate EMT, including the microRNA-200 family [25, 26]. The miR-200 family functions as a key regulator of EMT in numerous cancers and promotes epithelial phenotypes by directly targeting mRNA of E-cadherin transcriptional repressors ZEB1 and ZEB2 [27, 28]. Overexpression of micRNA-200c in A549 cells could suppress EMT by decreasing expression of Cathepsin L

Prognostic significance of the mir-200 family in lung cancer
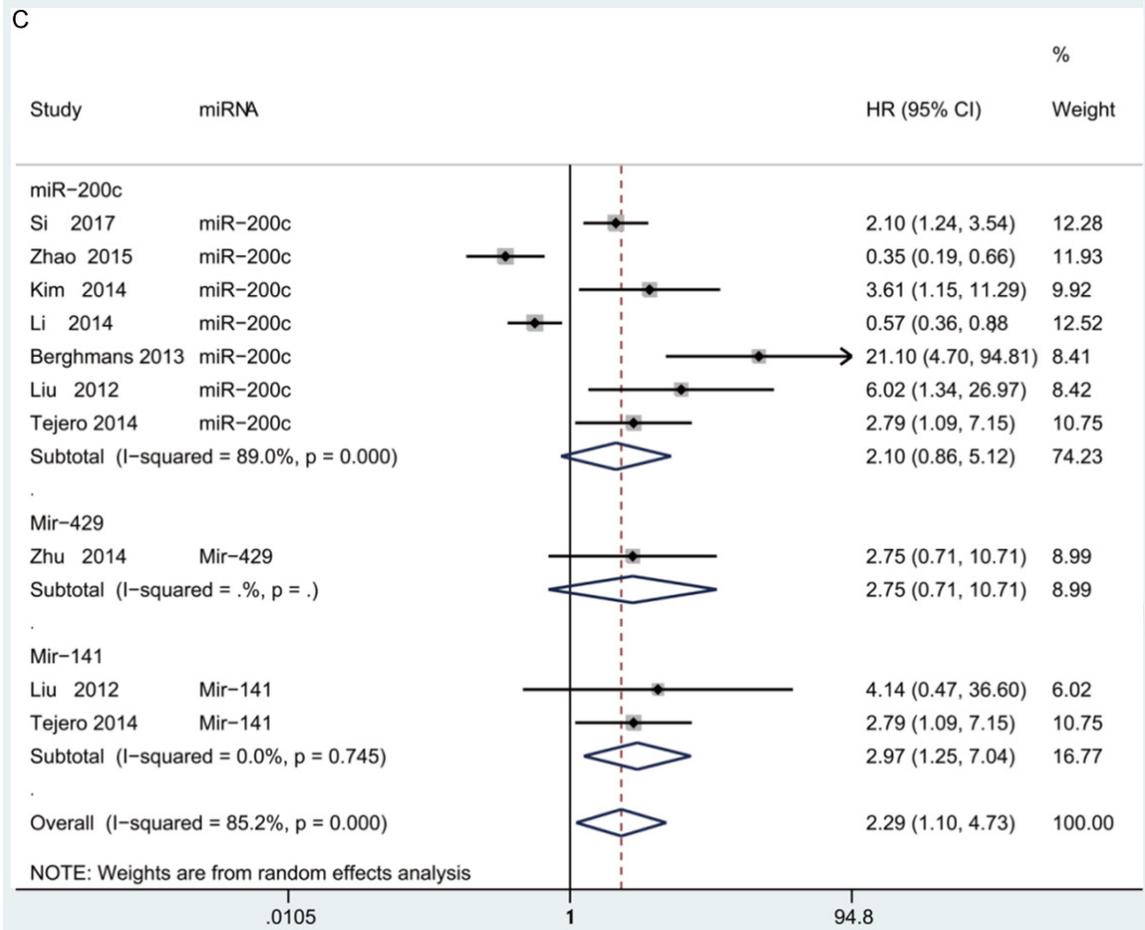
Figure 2. A. Forest plot of HRs for the relationship between miR-200 family overexpression and total OS. B. Forest plot of HRs for the relationship between miR-200 family overexpression and OS stratified by the area. C. Forest plot of HRs for the relationship between miR-200 family overexpression and OS stratified by the miRNA types.
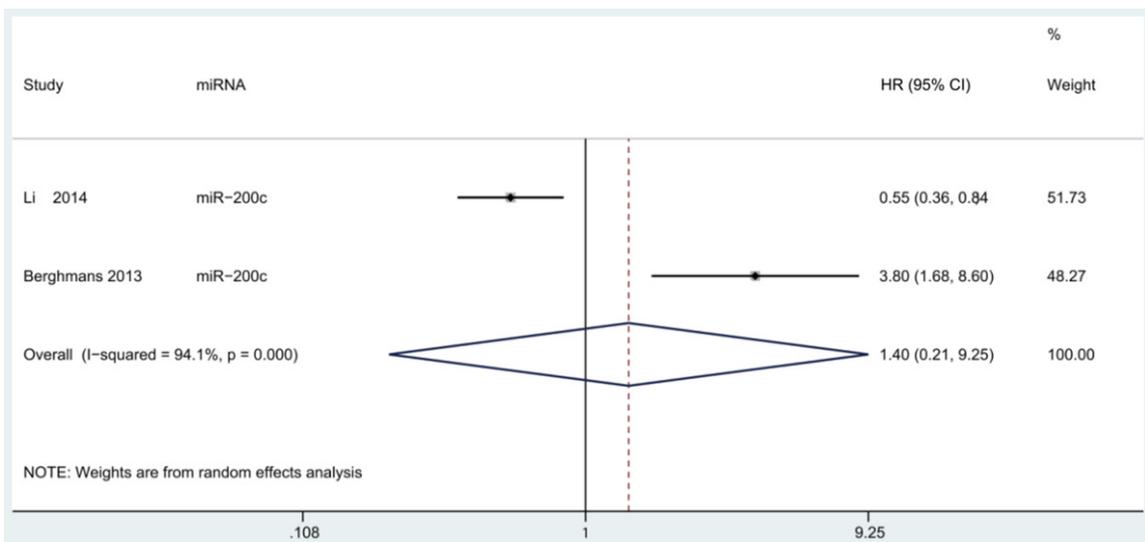

Figure 3. Forest plot of HRs for the relationship between miR-200 family overexpression and PFS.

Table 2. Meta-analysis results of the association between miR-200 family overexpression and clinico-pathological parameters

Clinicopathological parameter	Studies	Number of patients	OR (95% CI)	*P* value (95% CI)	*I²* (%)	P_h	Model
Age (>60 vs. <60 years)	5	477	1.118 (0.769-1.626)	0.558	39	0.161	Fixed-effects
Sex (male vs. female)	5	477	1.045 (0.708-1.542)	0.826	40.8	0.149	Fixed-effects
Histology (SCC vs. AD)	5	458	1.075 (0.733-1.577)	0.711	14.3	0.323	Fixed-effects
TNM stage (I-II vs. III-IV)	5	477	0.953 (0.608-1.491)	0.831	34.9	0.189	Fixed-effects
Lymph node metastasis (no vs. yes)	4	327	0.996 (0.427-2.320)	0.992	71.4	0.015	Random-effects
Tumor differentiation (well/moderate vs. poor)	4	327	1.159 (0.734-1.828)	0.527	42.3	0.158	Fixed-effects
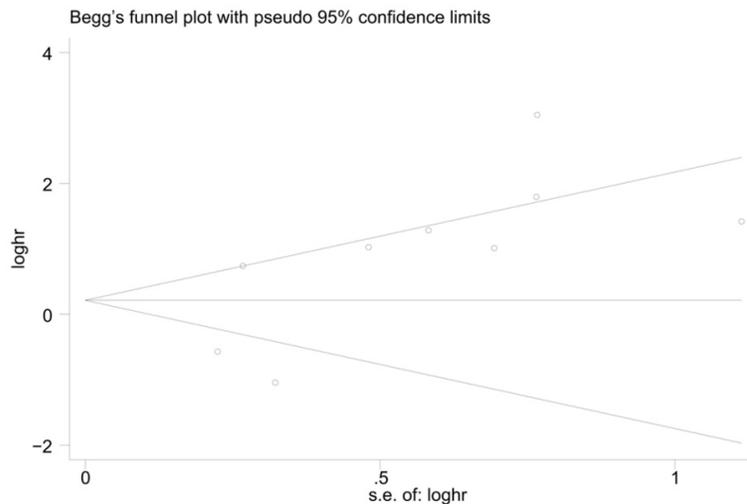
Figure 4. Publication bias results for OS.

(CTSL), a cysteine protease belonging to the papain-like family of cysteine proteinases. This protease plays important roles in tumor occurrence, development, metastasis, and chemotherapy resistance [29, 30]. CTSL has been recognized as a novel EMT regulator through its effects on expression of EMT-associated transcription factors, ZEB1, ZEB2, Snail, and Slug in human lung cancer cells [31]. In selecting another human lung cancer cell line, H1299 cells with wild-type KRAS, similar results were obtained, showing that micRNA-200c decreased CTSL expression [7]. In addition, one study noted that micRNA-200c could regulate paclitaxel resistance by inhibiting EMT. EMT was also reportedly associated with resistance to EGFR-TKIs in NSCLC patients. Li etc. [13] showed that miR-200c overexpression was associated with epithelial phenotypes and sensitivity to gefitinib in EGFR wild-type NSCLC cell lines. Upregulated miR-200c could regain sensitivity to gefitinib in the EGFR wild-type cell lines and miR-200c could regulate EMT through

PI3K/AKT and MEK/ERK pathways. They found that high levels of miR-200c expression were involved with longer OS and longer PFS, compared with the low miR-200c expression subgroup. However, Tejero et al. [19] found that high miR-200c and miR-141 expression was correlated with shorter OS in early-stage NSCLC adenocarcinoma. Apart from these, serum levels of miR-429 have been linked to poor overall survival of NSCLC patients. Both univariate and multivariate analyses have shown that serum miR-429 levels are an independent prognostic predictor for NSCLC [20]. Due to inconsistencies in those studies, the current meta-analysis shows that higher expression of miR-200 family is significantly associated with shorter OS of NSCLC patients. Results indicate that lung cancer cells could express more of the miR-200 family, which promotes tumor development and metastasis.

However, there were certain limitations to the current meta-analysis. First, pooled HRs were determined based on 11 studies with a small sample size of 812 patients. Second, there were a few minor heterogeneities in present meta-analysis results. It was found that the source of the heterogeneity originated from the area and subtype of the miR-200 family in meta-regression. In addition, the origin of heterogeneity was also probably due to differences in sample sizes and baseline patient characteristics (e.g age, cancer type, tumor stage, and treatment type). Stratified analysis by ethnicity and country supported the strong correlation of

miR-200 family and poor OS in Asian populations, with acceptable heterogeneity. Third, more than one study showed that higher expression of the miR-200 family was closely associated with TNM stage, positive lymph node metastasis, tumor differentiation, and so forth. However, this study failed to detect a relationship between overexpression of the miR-200 family and clinicopathological parameters. The reason may be insufficient research included. Fourth, many studies did not meet the inclusion criterion and were excluded, including some contradictory results. This may have caused a deviation of the results of this meta-analysis. Fifth, this meta-analysis did not evaluate the prognostic values of a combination of the miR-200 family and other miRNA markers for lung cancer. Due to limited data, further stratified analysis was unavailable. Therefore, larger, multi-centered, and higher-quality studies, with a unified criterion for determining miR-200 family expression, are necessary to validate present results.

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Disclosure of conflict of interest

None.

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