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Elevated miR-21 is associated with poor prognosis in non-small cell lung cancer: a systematic review and meta-analysis

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Abstract. – OBJECTIVE: Increasing studies have investigated the prognostic value of high miR-21 expression in non-small cell lung cancer (NSCLC) with inconsistent results. We conducted this meta-analysis to explore whether the expression of miR-21 was associated with prognosis in NSCLC patients.

MATERIALS AND METHODS: We systematically searched Medline, EMBASE, Web of Science and Cochrane Library for relevant studies. Studies exploring the relationship between miR-21 expression and NSCLC prognosis and clinical pathology, and reporting enough data to get the hazard ratio (HR) and 95% confidence intervals (CIs), were included. Random- or fixed-effect models were employed to calculated pooled hazard ratios (HRs) or risk ratio and 95% confidence intervals (95% CIs).

RESULTS: A total of 28 eligible studies, including 24 for prognosis, 16 for clinicopathological features were identified. Our results revealed that elevated miR-21 was related to unfavorable overall survival (OS) in NSCLC (HR = 1.960, 95% CI = 1.510-2.554, p = 0.000). Similar results were found in disease-free survival, relapse-free survival, and cancer-special death. In a meta-analysis of clinical pathology, overexpressed miR-21 was significantly related to lung adenocarcinoma, larger tumor size, and advanced clinical stage.

CONCLUSIONS: Our meta-analysis suggested that miR-21 may function as an unfavorable biomarker of prognosis in NSCLC patients.

Key Words:
microRNA-21, Non-small cell lung cancer, Prognosis, Meta-analysis.

Abbreviations

NSCLC: non-small cell lung cancer; HRs: hazard ratios; CIs: confidence intervals; OS: overall survival; miRNAs: microRNAs; 3′-UTRs: 3′-untranslated regions; mRNAs: messenger RNAs; miR-21: microRNA-21; CSD: cancer-special death; RFS: relapse-free survival; DFS: disease-free survival; PFS: progression-free survival; NOS: Newcastle-Ottawa Quality Assessment Scale; AD: adenocarcinoma; RR: risk ratio; SOCS1: suppressor of cytokine signaling 1; SOCS6: suppressor of cytokine signaling 6; PTEN: phosphatase and tensin homolog; PDCD4: programmed cell death 4.

Introduction

Lung cancer is the most common cancer, accounting for 12.9% (1.8 million) of the total newly diagnosed cancer cases globally[1]. It is also the leading cause of cancer-related mortality worldwide, estimated to be responsible for 1.59 million deaths (19.4% of the total). In spite of improvements in the treatment of lung cancer over the recent decades, the five-year survival rates are only 16.8%[2]. Approximately 85% of all lung cancers are classified as non-small cell lung cancer (NSCLC)[3]. Due to the lack of effective detection methods and the aggressive nature of this disease, most NSCLC patients have advanced-stage and incurable disease at the initial diagnosis[3]. The median survival rates for advanced NSCLC patients treated with histology-driven and/or maintenance, platinum-based chemotherapy only ranges from 10-13.9 mouths[4]. Early detection is crucial to prolonging survival, and the identification of molecular markers is key to predict prognosis and design novel managements for NSCLC.

MicroRNAs (miRNAs), approximately 18-25 nucleotides long, are a class of small evolutionarily conserved non-coding RNAs, involving regulating target genes expression at post-transcriptional level[5-9]. Although miRNAs

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do not encode protein themselves, they bind to 3′-untranslated regions (3′-UTRs) of target messenger RNAs (mRNAs), resulting in mRNA degradation or translational repression[10]. Previous investigations[11-13] have shown that miRNA could predict tumor classification, prognosis and responses of therapies. MicroRNA-21 (miR-21), transcribed by RNA polymerase II, promotes tumor cell proliferation, migration and invasions[14,15]. MiR-21 has been detected overexpressed in multiple malignancies including pancreatic cancer, esophageal cancer, colon cancer, and lung cancer[16-19].

Although increasing studies have investigated the prognostic significance of high miR-21 expression in NSCLC, the results have been inconsistent. For example, Wang et al[20] suggested that high miR-21 was associated with a poor prognosis in NSCLC; however, Voortman et al[21] found no correlation between miR-21 expression and overall survival (OS). To overcome the limitation of the single study, we conducted this systematic review to explore the prognostic value of miR-21 in NSCLC.

Materials and Methods

Search Strategy

We conducted a literature search using the databases including Medline, EMBASE, Web of Science and Cochrane Library from inception to 9 August 2017. The following terms were used: "lung cancer or lung carcinoma or lung neoplasm or lung adenocarcinoma or lung squamous cell carcinoma" and "miR-21 or miRNA-21 or miRNA-21 or miR21 or miRNA21 or miRNA21" and "prognosis or prognostic or survival". The reference lists of included studies were also examined for additional trails. Two reviewers independently screened the titles and abstracts of all retrieved records to exclude irrelevant studies. The remaining studies were assessed by reading full-text. Any disagreement was resolved by consensus or by involving an arbiter.

Inclusion and Excluded Criteria

The inclusion criteria of this review were: (1) studies exploring the relationship between miR-21 expression and NSCLC prognosis and clinical pathology; (2) studies reporting enough data to get the hazard ratio (HR) and 95% confidence intervals (CIs); (3) studies published in English. Studies were excluded for: (1) duplicate studies; (2) case reports, letters, reviews, conference abstracts, animal experiments and expert opinions; (3) studies with insufficient survival data; (4) not published in English.

Data Extraction and Methodological Quality Assessment

Two reviewers independently screened all included studies to extract the following data: name of the first author, publication year, country of the study, duration, follow-up, sample size, histology, ages, stage, cut-off value, method of detection, specimen, survival outcomes, analysis, HR and 95% CIs of miR-21 expression for OS, CSD, RFS, DFS, and PFS. Only HRs and 95% CIs of multivariate analysis were abstracted when univariate and multivariate analysis were both provided. If only Kaplan-Meier curves were presented in the studies, we utilized Engauge Digitizer version 4.3 to obtain the survival data, and Tierney's method to calculate the HRs and 95% CIs[22]. The Newcastle-Ottawa Quality Assessment Scale (NOS) was employed to assess the quality of each included studies. There are 9 items in NOS including three aspects of selection, comparability and outcome. NOS scores ≥ 6 were considered as high-quality studies. Any discrepancy will be resolved by discussion or by involving an arbiter.

Statistical Analysis

The HR and 95% CIs was employed to evaluate the prognostic efficiency of miR-21 on NSCLC. The overall HR > 1 and 95% CIs not overlapping in the forest plot indicated that NSCLC patients with elevated miR-21 had a poor prognosis, and HR < 1 and 95% CIs not overlapping in the forest plot implied a better survival. Assessment of heterogeneity was performed using Cochran's Q test and Higgins's I^2 [23], $I^2 < 50\%$ and p-value > 0.10 suggesting no heterogeneity. In absence of heterogeneity, a fixed-effects model was used. Otherwise, the random-effects model was applied[24]. Sensitivity was conducted by sequential omitting each study to examine the robustness of the results. Potential publication bias was evaluated by Begg's funnel plot and Egger's test[25]. If significant publication bias existed, trim and fill method was performed to validate the robust of the meta-analysis results[26]. All statistical analyses were calculated via Stata 13.0 (StataCorp, College Station, TX, USA). All two-tailed p-value < 0.05 was defined as statistically significance, except those for heterogeneity.

Results

Selection Process of Included Studies

As shown in the flow diagram (Figure 1), 522 articles were retrieved from Medline, EMBASE, Web of Science, and Cochrane Library databases. After the removal of duplicated articles, 373 articles were left. Next, we screened the titles and abstracts, 207 irrelevant articles were excluded. Subsequently, 166 of full-text articles were assessed for eligibility. 138 articles were excluded for the following reasons: 22 review or meta-analysis, 8 insufficient data, 34 conference abstracts, 38 not focus on survival, 18 not miR-21, 8 not NSCLC, 1 not in English, 7 animal or cell experiments, and 2 overlapped population. Among these, three articles[27-29] involving overlapped population, only the most recently published article (Robles, A. I. 2015) was included. Finally, 28 studies, 24 for prognosis and 16 for clinicopathological, were included in this meta-analysis.

Characteristics of Included Studies

Together, 24 eligible studies with a sample size of 3118 were used for analysis of prognosis, while 16 studies with 2131 patients were employed for analysis of clinicopathology. The main characteristics of eligible articles for prognosis were listed in Table I[20,21 27,30-50]. The Newcastle-Ottawa Scale (NOS) was used for quality assessment, and the NOS scores ranged from 5 to 9 (Table II). Among all cohorts, China (n=21) was the main source region, followed by USA (n=2) and Japan (n=2). Among 24 studies, 18 cohorts focused on primary outcome (OS), 13 cohorts focused on secondary outcomes: 5 for

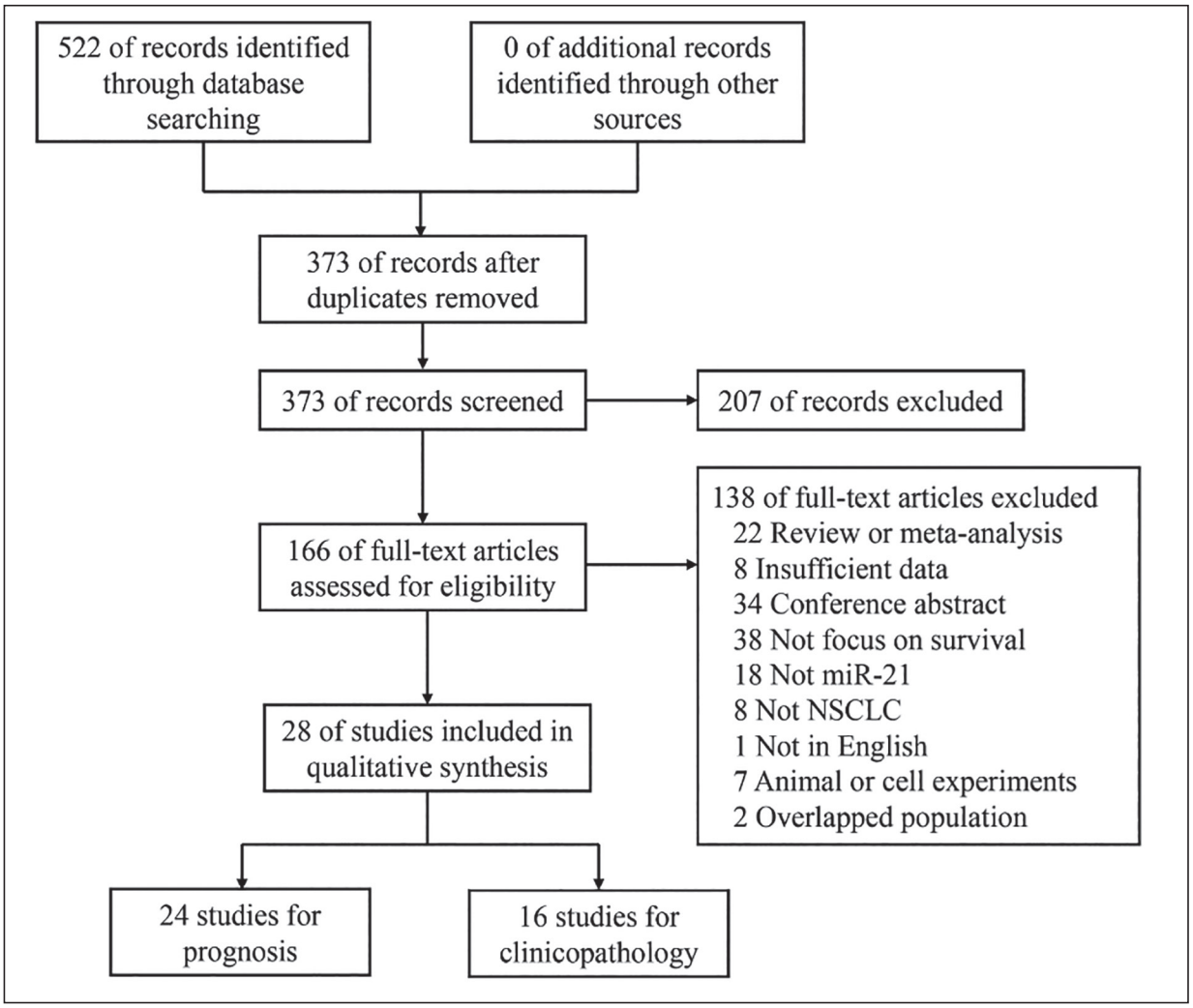

Figure 1. Flow diagram of study selection process.

Table I. Characteristics of the included studies used for survival analysis.

Study	Country	Duration	Follow up, mouths (Range)	Sample	Histology	Age, years (Range)	Stage	Cut-off value	Method	Specimen	Survival outcomes	Analysis	NOS
Dejima 2017[30]	Japan	2012.7-2015.7	Median 20 (0.5-37)	195	NSCLC	Mean 71 (32-88)	I-III	median	qRT-PCR	Plasma	DFS	M	8
Liu 2017[31]	China	2012.10-2014.12	Median 14.4 (3.43-36.87)	196	NSCLC	Mean ± SD 58.49 ± 9.95	I-IV	NA	qRT-PCR	Plasma	OS	M	8
Lin 2015[32]	Taiwan	1998-2004	NA	124	NSCLC	> 65 (65), ≤ 65 (59)	I-III	median	qRT-PCR	Tissues	OS, RFS	K-M	7
Begum 2015[33]	USA	NA	Mean 46.3 (1-204)	114	NSCLC	65.7	I-IV	1.297	qRT-PCR	Tissues	OS, RFS	U	7
Robles 2015 a[27]	USA/ Norway	1999-2012/ 1988-2003	NA	91	AD	Range (32-88)	I	NA	NanoString	Tissues	CSD	M	5
Robles 2015 b[27]	Japan	1998-2008	NA	113	AD	Range (39-76)	I	NA	NanoString	Tissues	RFS	M	5
Wang 2013[34]	China	2001-2007	NA	60	NSCLC	≥ 60 (38), < 60 (22)	I-IV	median	qRT-PCR	Tissues	OS	M	8
Le 2012[35]	China	2008.1-2009-12	NA	82	NSCLC	59	I-IV	0.85	qRT-PCR	Serum	OS	U	7
Liu 2012[36]	China	2008.1-2008.5	24	70	NSCLC	≥ 60 (36), < 60 (34)	I-IV	median	qRT-PCR	Tissues	OS	U	9
Gao 2012[37]	China	2009.1-2009.10	Up to 2010.11	58	NSCLC	≥ 60 (25), < 60 (33)	I-III	NA	qRT-PCR	Tissues	DFS	M	8
Wang 2011[38]	China	2003-2005	52.16 (1–73)	88	NSCLC	Median 57 (40–71)	I-III	5	qRT-PCR	Serum	OS	M	9
Markou 2008[39]	Greece	2004-2005	39	48	NSCLC	≥60(25), <60(23)	I-IV	2	qRT-PCR	Tissues	OS, DFS	M	9
Wang 2017[20]	China	2008.1-2014.12	Up to 2015.12	216	NSCLC	Mean ± SD 55.9± 15.1	I-III	4.49	qRT-PCR	Tissues	CSD	M	7

Continued

Table I *(Continued)*. Characteristics of the included studies used for survival analysis.

Study	Country	Duration	Follow up, mouths (Range)	Sample	Histology	Age, years (Range)	Stage	Cut-off value	Method	Specimen	Survival outcomes	Analysis	NOS
Cinegaglia 2016[40]	Brazil	NA	NA	17	AD	Median 66.8 (43-83)	I-IV	median	qRT-PCR	Tissues	OS	NA	5
Tian 2016[41]	China	2001.3-2007.12	46.7 (5.0–71.0)	204	NSCLC	Median 47.9 (25– 84)	I-III	5	qRT-PCR	Tissues	OS, PFS	M, K-M	7
Stenvold 2014[42]	Norway	1990-2004	Median 105 (73-234)	335	NSCLC	Median 67 (28-85)	I-IIIA	NA	ISH	Tissues	CSD	M	8
Gao 2011[43]	China	2014.2-2015.1	Up to 2009.9.30	30	SCC	> 63 (15), ≤ 63 (15)	I-III	1.47	qRT-PCR	Tissues	OS	M	7
Gao 2010[44]	China	2004.2-2006.1	Up to 2009.7.28	47	NSCLC	> 64 (23), < 64 (24)	I-III	1.4	qRT-PCR	Tissues	OS	M	8
Li 2017[45]	China	2007.11-2010.3	Up to 2012.12.31	78	NSCLC	Median 63.5 (39-78)	I-IV	median	qRT-PCR	Tissues	OS	M	8
Voortman 2010[21]	14 countries	NA	8	631	NSCLC	< 55 (191), 55-64 (272), > 64 (168)	I-III	median	qRT-PCR	Tissues	OS	NA	8
Capodanno 2013[46]	Italy	2005-2012	32 (7-98)	80	NSCLC	Median 67 (46-85)	I-IV	6.964	qRT-PCR	Tissues	OS, PFS	K-M	7
Shen 2014[47]	China	NA	NA	47	NSCLC	Mean 63.98 (44~80)	I-IV	median	qRT-PCR	Tissues	DFS	K-M	6
Zhao 2015[48]	China	2012.1-2013.12	12-48	80	NSCLC	Mean 57.61 (44-71)	NA	1.21	qRT-PCR	Serum	OS	K-M	8
Xue 2016[49]	China	NA	NA	80	NSCLC	NA	I-III	median	qRT-PCR	Tissues	OS, DFS	K-M	5
Yang 2015[50]	China	1999.1-2007.8	NA	34	NSCLC	NA	NA	NA	ISH	Tissues	OS	K-M	5

Abbreviations: NA, not applicable; NSCLC, non-small cell lung cancer; AD, adenocarcinoma; SCC, squamous cell carcinoma; qRT-PCR, quantitative real-time polymerase chain reaction; ISH, In situ hybridization; OS, overall survival; DFS, disease-free survival; RFS, relapse-free survival; CSD, cancer-special death; PFS, progression-free survival; NOS, Newcastle-Ottawa Quality Assessment Scale, M, Multivariate analysis; U, Univariate analysis; K-M, Kaplan-Meier survival curves.

Table II. Quality indicators from the Newcastle-Ottawa scale.

Study	Selection			Comparable			Outcome assessment			Score
	1	2	3	4	5	6	7	8	9	
Dejima, H. 2017	*	*	*	*	*	*	*	*		8
Liu, Q. Y. 2017	*	*	*	*	*	*	*	*		8
Lin, T. C. 2015	*	*	*		*	*	*	*		7
Begum, S. 2015	*	*	*		*	*	*	*		7
Robles, A. I. 2015 a		*	*		*		*	*		5
Robles, A. I. 2015 b		*	*		*		*	*		5
Wang, X. C. 2013	*	*	*	*	*	*	*	*		8
Le, H. B. 2012		*	*	*	*	*	*	*		7
Liu, X. G. 2012	*	*	*	*	*	*	*	*	*	9
Gao, W. 2012	*	*	*	*	*	*	*	*		8
Wang, Z. X. 2011	*	*	*	*	*		*	*	*	8
Markou, A. 2008	*	*	*	*	*	*	*	*	*	9
Wang, X. 2017	*	*	*		*	*	*	*		7
Cinegaglia, N. C. 2016		*	*		*		*	*		5
Tian, L. 2016	*	*	*		*	*	*	*		7
Stenvold, H. 2014	*	*	*		*	*	*	*	*	8
Gao, W. 2011	*	*	*		*	*	*	*		7
Gao, W. 2010		*	*	*	*	*	*	*		7
Li, Y. W. 2017	*	*	*	*	*	*	*	*		8
Voortman, J 2010	*	*	*	*	*	*	*	*		8
Capodanno, A. 2013	*	*	*		*	*	*	*		7
Shen, H. 2014	*	*	*		*		*	*		6
Zhao, W. 2015		*	*	*	*	*	*	*	*	8
Xue, X. Y. 2016		*	*		*		*	*		5
Yang, Z. H. 2015		*	*		*		*	*		5
Chiou, Y. H. 2015	*	*	*		*		*	*		6
Zhao, Q. 2015	*	*	*		*		*			5
Yang, J. S. 2015	*	*	*		*	*	*			6
Ye, M. 2017	*	*	*		*	*	*			6

disease-free survival (DFS), 3 for relapse-free survival (RFS), 3 for cancer-special death (CSD), and 2 for relapse-free survival (PFS). Besides, 16 studies focused on clinicopathology.

Meta-Analysis of OS

18 studies with 2063 patients were included in the meta-analysis of OS. Due to significant heterogeneity (I^2 =77.2, p=0.000), the random-effect model was employed. The result revealed that elevated miR-21 was expected to predict unfavorable OS when compared with the low expressed miR-21 in NSCLC (Figure 2, hazard ratio (HR)=1.960, 95% CI=1.510-2.544, p=0.000). In view of heterogeneity, subgroup analyses were conducted according to the potential confounders, such as study region, clinical stage, sample size, analysis method, specimen, and cut-off of miR-21 (Table III). When stratified by study region, only studies conducted in China showed that high expressed miR-21 was associated with poor OS, with significant heterogeneity (HR=2.101, 95% CI=1.581-2.793, p=0.000; I^2=68.9%, random-effect model). As for the subgroup analysis of clinical stage, patients with high expressed miR-21 were expected to suffer unfavorable OS in stage I-III (HR=1.827, 95% CI=1.203-2.777, p=0.005; I^2=90.1%, random-effect model), and similar result was observed in stage I-IV (HR=2.069, 95% CI=1.598-2.680, p=0.000; I^2=0.0%, fixed-effect model). As for subgroup analysis of sample size, there was not association observed between elevated miR-21 and OS in studies with sample size > 100 (HR=1.747, 95% CI=0.933-3.272, p=0.081; I^2=91.1%, random-effect model), while the result in studies with sample size < 100 showed that elevated miR-21 was an unfavorable factor for prognosis (HR=2.064, 95% CI=1.566-2.719, p=0.000; I^2=53.9%, random-effect model). As for studies assessed by multivariate analysis, the result revealed that high miR-21 was significantly related to poor OS (HR=2.315, 95% CI=1.590-3.370, p=0.000; I^2= 81.3%, ran-

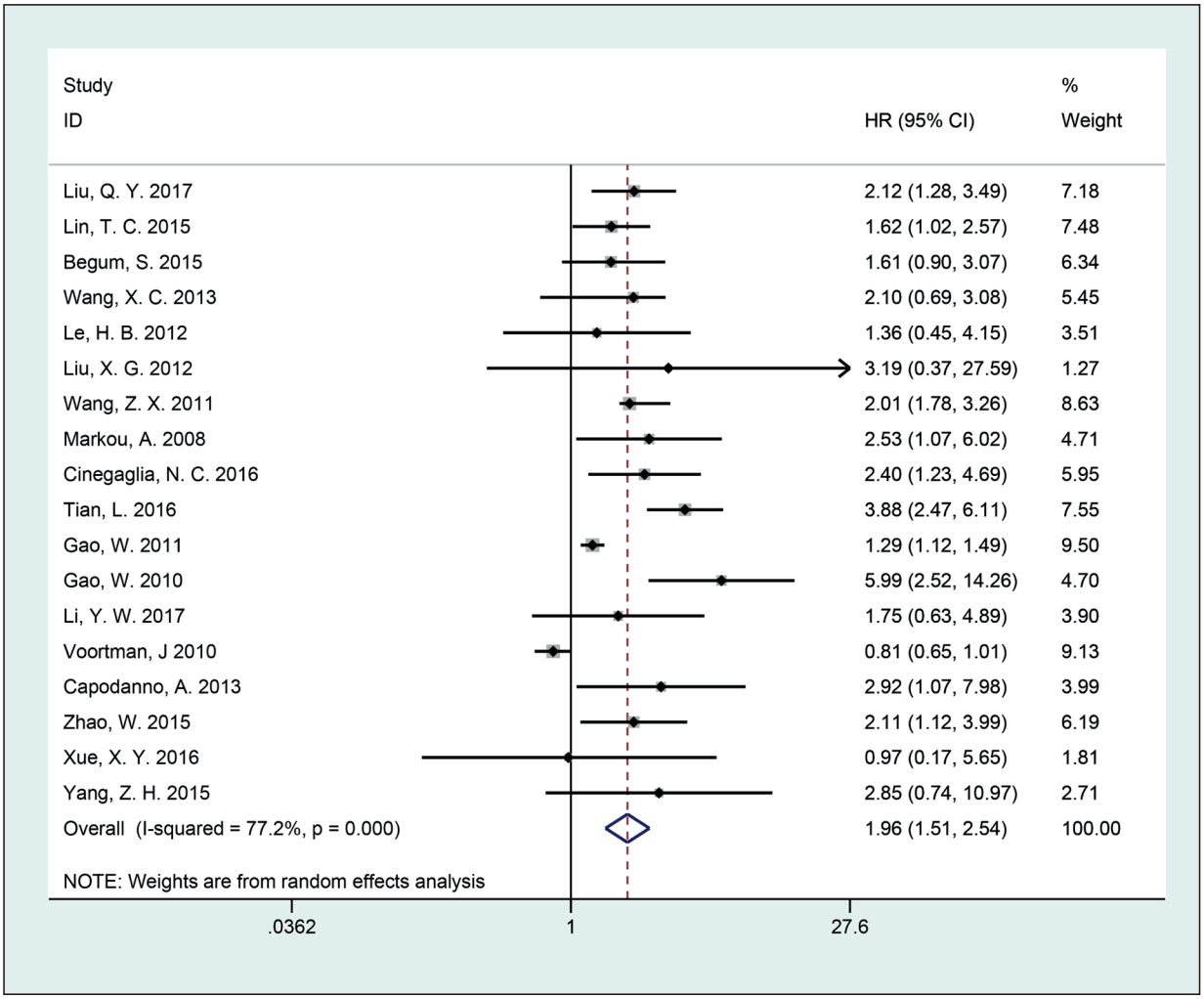

Figure 2. The correlation between miR-21 and overall survival in non-small cell lung cancer.

dom-effect model). Similarly, significant association was observed between elevated miR-21 and poor OS in studies with datum extracted from Kaplan-Meier survival curves (HR=1.888, 95% CI=1.354-2.634, p=0.000; I^2=0.0%, fixed-effect model). However, a similar result was not observed in the researches assessed by univariate analysis. As for subgroup analysis of specimen, Liu et al[36] examined miR-21 expression in both blood and tissue, and we divided them into subgroup of blood and tissue, respectively. High expressed miR-21 was significantly associated with poor OS in blood group (HR= 2.057, 95% CI=1.632-2.592, p=0.000; I^2=0.0%, fixed-effect model), and similar result was seen in group of tissue (HR=1.985, 95%CI=1.435-2.746, p=0.000; I^2=79.9%, random-effect model). As for subgroup analysis of cut-off of miR-21, elevated miR-21 was expected to predict poor OS in median group (HR=1.542, 95% CI=1.151-2.065, p=0.004; I^2=68.3%, random-effect model). A similar result was seen in group of non-median (HR=2.544, 95% CI=1.712-3.782, p=0.000; I^2= 60.2%, random-effect model).

Meta-Analysis of DFS/RFS/CSD/PFS

5 studies reporting DFS, 3 reporting RFS, 3 reporting CSD and 2 covering PFS were included into this meta-analysis (Figure 3). Significant association was observed between elevated miR-21 and DFS (HR=2.154, 95% CI=1.281-3.624, p=0.004; I^2=0.0%, fixed-effect model), RFS (HR=1.693, 95% CI=1.176-2.437, p=0.005; I^2=0.0%, fixed-effect model), and CSD (HR=1.002, 95% CI=1.001-1.003, p=0.000; I^2=32.9%, fixed-effect model). PFS was not related to miR-21.

Table III. The main results of subgroup analysis.

Analysis	Category	Study (n)	Model	HR(95%CI)	Z	p	Heterogeneity	
							I^2	P_h
Study region	China	13 (1173)	Random	2.101 (1.581-2.793)	5.11	0.000	68.9%	0.000
	Other countries	5(890)	Random	1.736 (0.940-3.207)	1.76	0.078	81.0%	0.000
Clinical stage	I-III	7(1204)	Random	1.827 (1.203-2.777)	2.82	0.005	90.1%	0.000
	I-IV	9(745)	Fixed	2.069 (1.598-2.680)	5.51	0.000	0.0%	0.971
Sample size	>100	5(1269)	Random	1.747 (0.933-3.272)	1.74	0.081	91.1%	0.000
	<100	13(794)	Random	2.064 (1.566-2.719)	5.15	0.000	53.9%	0.011
Analysis method	Multivariate analysis	8(751)	Random	2.315 (1.590-3.370)	4.38	0.000	81.3%	0.000
	Univariate analysis	3(226)	Fixed	1.615 (0.959-2.721)	1.80	0.072	0.0%	0.790
	K-M survival curves	5(398)	Fixed	1.888 (1.354-2.634)	3.74	0.000	0.0%	0.703
Specimen	Blood	5(516)	Fixed	2.057 (1.632-2.592)	6.12	0.000	0.0%	0.789
	Tissue	14(1617)	Random	1.985 (1.435-2.746)	4.14	0.000	79.9%	0.000
Cut-off of miR-21	Median	10(1250)	Random	1.542 (1.151-2.065)	2.91	0.004	68.3%	0.001
	Non-median	6(583)	Random	2.544 (1.712-3.782)	4.62	0.000	60.2%	0.028

Abbreviations: P denotes *p* value for statistical significance based on Z test; Ph denotes *p* value for heterogeneity based on Q test; HR, hazard ratio; CI, confidence interval.

Meta-Analysis of Clinicopathology

There are 13 trials with 1920 patients reported the correlation between miR-21 and histology, and the pooled outcome indicated that high miR-21 was related to adenocarcinoma (AD, risk ratio (RR)=1.157, 95% CI: 1.051-1.273, p=0.003; I^2=39.4%, fixed-effect model). The relationship of miR-21 and tumor size was reported in 10 studies with 1630 patients, and a significant association was seen between elevated miR-21 and larger tumor size (RR=1.169, 95% CI: 1.041-1.312, p=0.008; I^2=26.2%, fixed-effect model). 10 studies with 1660 patients reported correlation between miR-21 and stage, and the conjoined result declared that high miR-21 was significantly related to stage III+IV (RR=1.401, 95% CI: 1.137-1.728, p=0.002; I^2=75.9%, random-effect model). However, miR-21 was not significantly associated with age, gender, smoking, epidermal growth factor receptor (EGFR), lymph node metastasis, lymphatic invasion and differentiation (Table IV).

Sensitivity Analysis and Publication Bias

Sensitivity analysis was conducted by sequential omitting each study to assess the robustness of OS. Result suggested that no point estimate of

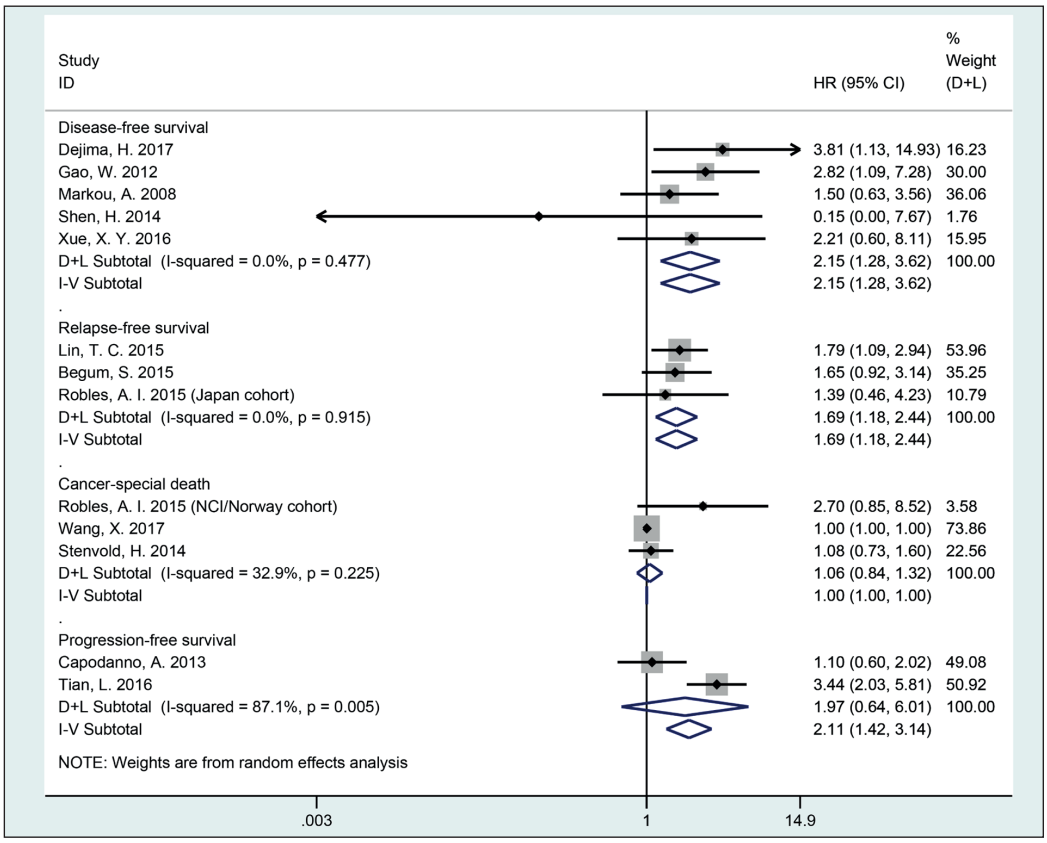

Figure 3. The correlation between miR-21 and disease-free survival, relapse-free survival, cancer-special death and progression-free survival in non-small cell lung cancer.

Table IV. Summary of the association of miR-21 and clinopathological parameters in non-small cell lung cancer

Category	Study (n)	Model	RR(95%CI)	Z	p	Heterogeneity	
						I^2	P_h
Age (>65 vs. ≤65)	14(1890)	Fixed	0.939(0.850-1.037)	1.25	0.213	24.7%	0.188
Gender (Male *vs*. Female)	15(2083)	Fixed	1.028(0.934-1.131)	0.56	0.578	20.1%	0.230
Histology (AD *vs*. non-AD)	13(1920)	Fixed	1.157(1.051-1.273)	2.99	0.003	39.4%	0.071
Smoking (Yes *vs*. No)	11(1093)	Random	0.853(0.695-1.047)	1.52	0.129	56.6%	0.011
EGFR (+ *vs*. -)	3(453)	Random	0.588(0.174-1.992)	0.85	0.394	91.8%	0.000
Tumor size (≥3 cm vs. <3 cm)	10(1630)	Fixed	1.169(1.041-1.312)	2.64	0.008	26.2%	0.202
Lymph node metastasis (+ *vs*. -)	13(1878)	Random	1.203(0.942-1.538)	1.48	0.139	80.9%	0.000
Lymphatic invasion (+ *vs*. -)	3(884)	Fixed	1.164(0.999-1.357)	1.95	0.051	0.0%	0.835
Differentiation (Well or Moderate vs. poor)	7(754)	Fixed	0.906(0.767-1.071)	1.16	0.248	36.2%	0.152
Stage (III+IV *vs*. I+II)	10(1660)	Random	1.401(1.137-1.728)	3.16	0.002	75.9%	0.000

Abbreviations: *P* denotes *p* value for statistical significance based on *Z* test; P_h denotes *p* value for heterogeneity based on Q test; RR, risk ratio; CI, confidence interval; EGFR, epidermal growth factor receptor.

the omitted individual dataset lay outside the 95% CI of the pooled analysis based on the overall HR estimate of OS (Figure 4).

Begg's funnel plot and Egger's test were applied to evaluate the publication bias of the studies used for calculating OS. The funnel plot was asymmetrical. The *p*-value calculated from Egger's test suggested the presence of publication bias (p=0.016) among these studies (Figure 5). The trim and fill analysis showed that 8 non-published studies were needed to balance the funnel plot (Figure 6). The adjusted HR and 95% CI attenuated but remains significant (pooled HR=1.376, 95% CI=1.067-1.773, p=0.014, random effects), thereby suggesting that the potential publication bias had minimal impact on the overall outcome.

Discussion

MiR-21 is one of the most highly expressed miRNA, which can promote tumor progression according to downregulate the expression of suppressor of cytokine signaling 1 (SOCS1), suppressor of cytokine signaling 6 (SOCS6), phosphatase and tensin homolog (PTEN) and programmed cell death 4 (PDCD4)[49,51]. Many researches have focused on the prognostic value of miR-21 in NSCLC but with contradictive results. Thus, we conducted this meta-analysis to comprehensively assess the survival value miR-21 in NSCLC, hoping to draw a proper conclusion.

In our study, results suggested that patients with elevated of miR-21 had a poor OS compared with the others with low expressed miR-21 in NSCLC. In subgroup analysis of study region, the correlation between high miR-21 and unfavorite OS was only observed in studies conducted in China. In the group of other countries, mostly studies (4/5) revealed that high miR-21 was related to shorter OS except the investigation of Voortman et al[21] (HR=0.81, 95% CI=0.650-1.010), which was conducted in 14 countries with 631 patients. However, Gallach et al[19] and Markou et al[52] detected that high miR-21 was an unfavorable factor for OS in Spain ($p < 0.0001$) and Greece (p=0.037), but were excluded from the meta-analysis for only reporting the relevant

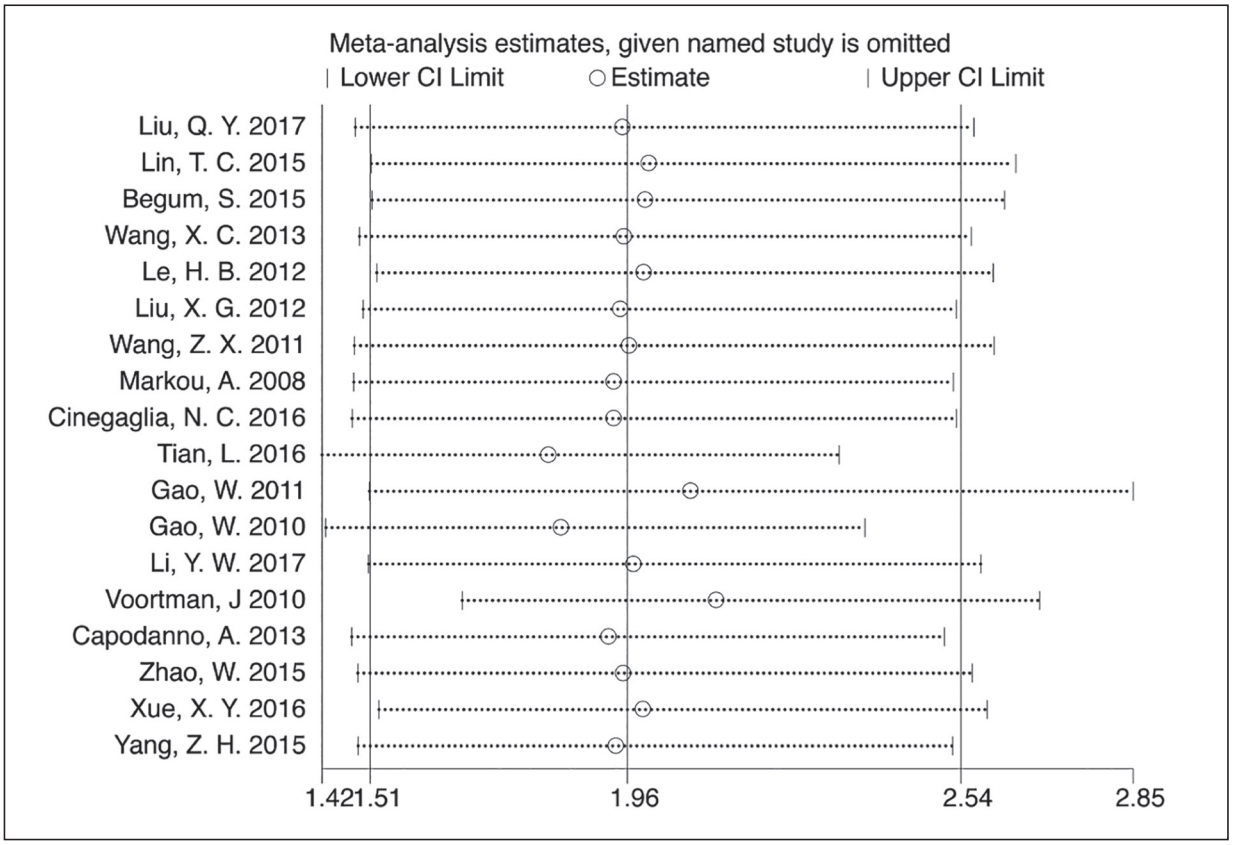

Figure 4. Sensitivity analysis of the influence of each individual study on the pooled hazard ratios (HRs) for the relationship between miR-21 and overall survival by omitting individual studies.

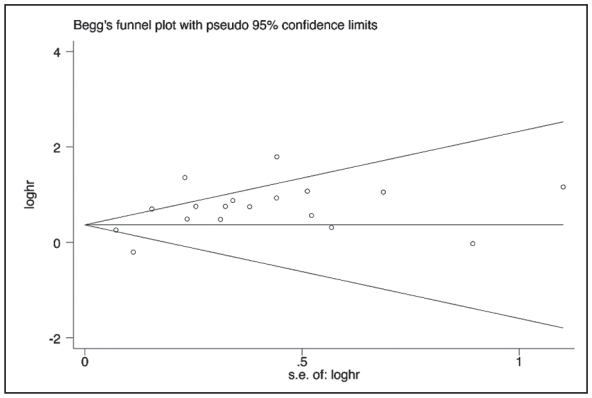

Figure 5. Funnel plot of publication bias for studies reporting overall survival.

p-value. Therefore, more cohort studies should be carried out to explore the prognostic value of miR-21 in NSCLC in countries other than China. Five studies were included in subgroup of sample size > 100 with a negative result (HR=1.747, 95% CI=0.933-3.272, p=0.081). The lower limit of 95% CI (0.933) was close to 1. Maybe more studies carried out with sample size > 100 will get a positive outcome of correlation between high miR-21 and poor OS.

Patients with high expressed miR-21 examined whether in blood or in tissue, were expected to suffer a shorter OS. To our knowledge, miRNAs may enter circulation mainly from microvesicles/exosomes derived from tumor cell[53,54]. Microvesicles, shed from many cell types, can transfer miRNA to other cell types and generate the similar function[55,56]. And study had shown that some miRNAs was overexpressed in microvesicles shed from tumor cells than inside the cells[56].

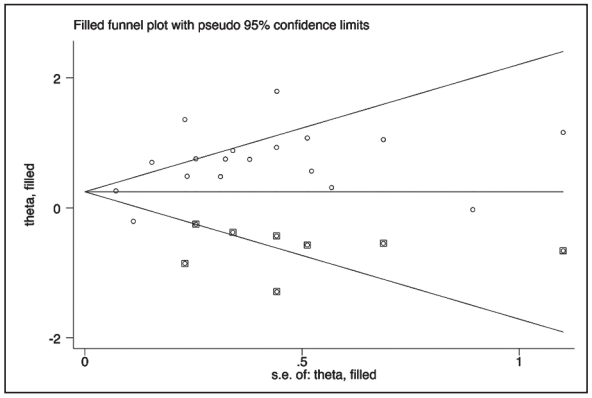

Figure 6. Funnel plot adjusted with trim and fill method for studies reporting overall survival.

However, Hu et al[57] reported that some miRNAs, which overexpressed in lung cancer tissue, were not detectable in the serum. The results from Hu et al[57] might suggest that the predictive role of serum miRNAs could be independent from tissue specimen. Our meta-analysis suggested that miR-21 not only in tissue but also in circulation was a significant prognostic biomarker. Otherwise, as a noninvasive and easily detected biomarker in circulation, miR-21 may be used to investigate the response of therapies in NSCLC. Zhu et al[58] found that overexpressed plasma miR-21 was predictive of insensitivity in patients with advanced lung AD to first-line pemetrexed and platinum-based chemotherapy. But the mechanisms of all the above results need to be additional explored.

In our study, significant association between elevated miR-21 and DFS, RFS and CSD was observed. However, similar result was not seen in PFS. The negative outcome of correlation between miR-21 and PFS might result from the fewer number of the included study and smaller sample size. More prospective cohort studies should be conducted to explore whether high miR-21 predict poor PFS in NSCLC.

Synthesized data of association between miR-21 and clinicopathology features indicated that overexpressed miR-21 was significantly related to AD, larger tumor size and advanced clinical stage. Our results were consistent with study conducted by Liu et al[36] which was excluded from meta-analysis of clinicopathology due to insufficient data.

Four previous articles[59-62] have explored the value of miR-21 expression in prognosis of NSCLC, and concluded that elevated miR-21 was associated with poor OS in NSCLC. Novelty of our meta-analysis are threefold. First, in the four meta-analyses, not only NSCLC but also other cancers (breast cancer, colorectal cancer, pancreatic cancer and so on)[60] were included and other miRNAs (miR-155, miR-126, miR-200c and so on)[59, 61, 62] were included. With other cancers and miRNAs, most of them only explore the relationship between miR-21 and OS in NSCLC. Our review only included studies involving miR-21 and NSCLC, and explored more prognostic indicators, like DFS, CSD, RFS and PFS. Our review firstly found that overexpressed miR-21 is associated with poor DFS, RFS and with higher CSD (Figure 3). Moreover, comparing Chinese people and other countries' people, we found that elevated miR-21 related to shorter OS is more suitable for Chinese people. (Table III) Second,

we conducted meta-analysis between miR-21 and clinicopathology in NSCLC firstly, and found that overexpressed miR-21 was significantly associated with AD, larger tumor size and advanced clinical stage. (Table IV) Larger tumor size, and advanced clinical stage are common variables associated with tumor progression. Therefore, NSCLC patients, especially AD patients, with these factors would benefit most from evaluation of miR-21 expression to make clinical decisions. Third, lots of important studies involving miR-21 and prognosis in NSCLC were not included in the four meta-analysis. With more reasonable search strategy, we included 24 studies to explore the value of miR-21 expression in prognosis of NSCLC. However, Zhan et al[59] only included 7 studies, Zhu et al[60] including 3, Yang et al[61] including 5 and Wang et al[62] including 7. Overall, with expanded prognostic indicators (including OS, DFS RFS, CSD, PFS), clinicopathology, and an updated inclusion of recent studies, which were not included by the four meta-analyses, our study (3118 patients for prognosis, 2131 patients for clinicopathology) is responsible for a more robust conclusion with a larger sample size. There are several potential limitations in our meta-analysis. Firstly, publication bias was detected according to Begg's funnel plot and Egger's test in our meta-analysis. Publication bias might result from: studies with positive results were more likely to be published than negative results; only studies published in English were included in this meta-analysis. The trim and fill analysis was performed to validate the robust of the meta-analysis results. However, different conclusions did not appear with and without trim and fill analysis. Secondly, some studies did not provide HRs and 95% CIs of multivariate analysis, we had to calculate HRs and 95% CIs from Kaplan-Meier curves or univariate analysis, which might slightly be different from the actual HRs. Thirdly, heterogeneity existed across studies. A random-effect model was employed to take variation into consideration, and we carried out subgroup analysis to control the influence of the heterogeneity. Sensitivity analysis suggested that no point estimate of omitted individual study lay outside the 95% CI of the pooled analysis.

Conclusions

Our meta-analysis revealed that elevated miR-21 is an unfavorable predictor of OS, DFS, RFS and CSD in NSCLC. Moreover, overexpressed miR-21 was significantly related to AD, larger tumor size and advanced clinical stage. Therefore, high miR-21 is a promising prognostic biomarker for NSCLC, especially for AD.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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