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Prognostic value of MicroRNA-21 in non-small cell lung cancer: a meta-analysis

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Abstract: **Recent studies have shown that microRNA-21 (miR-21) may act as a prognostic biomarker in non-small cell lung cancer (NSCLC). However, the available results remain controversial. Therefore, we performed a meta-analysis to identify the prognostic role of miR-21 in NSCLC. Eligible published studies were identified and assessed for quality through several search strategies. Data were extracted from each study to investigate the association between miR-21 and survival in NSCLC patients. With respect to survival outcomes, the hazard ratio (HR) with 95% confidence intervals was utilized to calculate pooled effect size. A total of nine articles involving 1639 cases were identified in this meta-analysis. The pooled HR suggested that a high miR-21 expression can significantly predict worse NSCLC survival (HR=2.29, 95%CI: 1.58-3.33 P<0.0001). Moreover, elevated miR-21 level was significantly correlation with lowered overall survival (OS) in the Asian group (HR=2.88 95%CI: 1.64-5.08 P<0.00001) than Caucasian cohort (HR=1.57 95%CI: 0.68-3.65 P=0.0002). Furthermore, subgroup analysis suggested that circulating miR-21 had a prognostic value in patients with NSCLC (HR=2.49 95%CI: 1.85-3.35 P<0.00001). This meta-analysis provides evidence that miR-21 can predict unfavorable prognosis in NSCLC.**

Keywords: MicroRNA-21; Prognosis; Non-small cell lung cancer; Meta-analysis

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1. Introduction

Lung cancer is the leading cause of cancer-related mortality in both men and women[1]. Non-small cell lung cancer (NSCLC) accounts for more than 80% of all cases, which is the main type of lung cancer. Approximately 60% of NSCLCs are diagnosed at advanced stages, resulting in the 5-year survival rate of only about 14%. Although the comprehensive regimes have been used to manage NSCLC, the prognosis for patient with advanced NSCLC remains unencouraging[2,3]. Therefore, we urgently need to exploit innovative and reliable prognostic or diagnostic biomarkers for the treatment of NSCLC. Recently, studies demonstrated that microRNAs (miRNAs) play a critical role in the development of NSCLC and could be applied in the early detection and prognosis of NSCLC[4].

MiRNAs, a class of endogenous small non-coding RNA molecules, are highly evolutionalily conserved, and influence a variety of human biological processes including metabolism, cell proliferation, differentiation, and apoptosis[5,6]. In addition, accumulating evidence has shown that miRNAs are aberrantly expressed in various tumors, and can considered as either oncogenes or suppressor genes depending on the functional classification[7-9].

MicroRNA-21 (miR-21) is the most extensively explored cancer-related micro-RNA. Previous studies have observed that miR-21 consistently dysregulates in lung cancer and acts as an oncogene mediating the growth, development, and progression of NSCLC[10,11]. More importantly, recent clinical studies found that a higher miR-21 expression was linked with the prognosis of lung cancer patients, but the results are controversial[12-14].

Therefore, it is timely and necessary to perform a meta-analysis to address the inconsistencies of the literature and evaluate the prognostic value of miR-21 for NSCLC.

2. Materials and Methods

2.1. Search strategy and study selection

We have collected the eligible studies by searching the PubMed, EMBASE, Cochrane library, Web of Science up to October 2016. The following keywords were used in various combinations:‘mircroRNA-21 or miRNA-21 or miR-21’ and ‘lung cancer’ , ‘lung neoplasms’ ,‘lung tumor’ ,‘non-small cell lung cancer or NSCLC’.

All of the studies which met the following criteria were considered eligible: (1) patients diagnosed lung cancer based on histopathological confirmation (2) the expression of miR-21 in the serum or tissue was detected (3) associations between miR-21 expression levels and survival outcomes were measured. Studies were excluded according to the following criteria: (1) basic research and reviews (2) studies lacked key information to calculate HR and 95% confidence intervals (CIs). Two reviewers (XF and MLN) select the qualified articles independently based on the eligibility criteria. Disagreement was resolved by consulting with a third investigator (YZ). A flow diagram of the study selection process is presented in Figure 1.

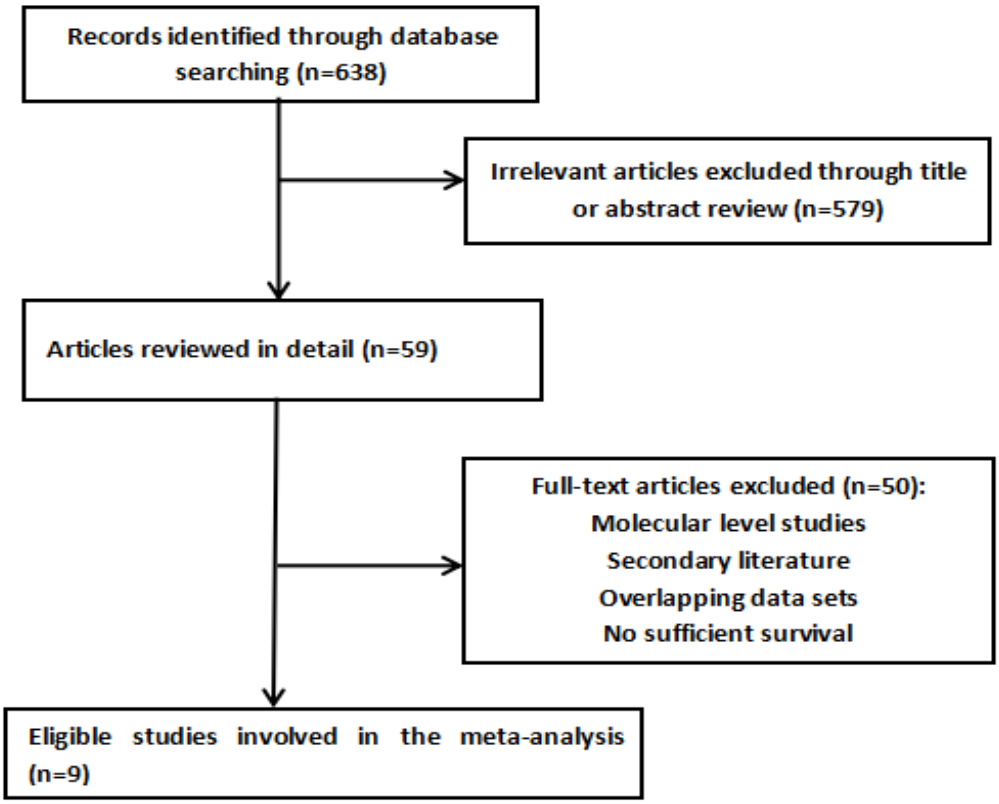

Figure 1. Flow chart of study selection.

2.2. Quality assessment

Following a critical review checklist of the Dutch Cochrane Centre proposed by MOOSE[15], we systematically assessed the quality of each eligible study. The key points to be evaluated are as follows: (i) clear description of the study population and ethnicity; (ii) clear definition of study design; (iii) clear definition of outcome assessment; (iv)clear explanation of measurement of miR-21; (v) period of follow-up was sufficient; (vi) clear definition of the cut-off value of miR-21. We excluded the articles that didn't mention all six points.

2.3. Data extraction

Two reviewers (XF and MLN) independently extracted the required information from all included studies. The data elements of this meta-analysis including the following aspects: (i) first author, published year, study design; (ii) the characteristics of the studied population, including the ethnicity, sample size, sample type, TNM stage, detection method. (iii) miR-21 expression levels and cut-off values. (iv) statistical data including the HR of elevated miR-21 for overall survival (OS), relapse-free survival (RFS) or disease-free survival (DFS), as well as their 95%CI and P values. If HR and 95%CI were not provided, we calculated these values using a previous described method by Parmar MK[16].

2.4. Statistical analysis

The HRs and 95%CIs extracted from the eligible studies were pooled for the survival results. Statistical heterogeneity of the combined HRs was assessed by the chi-square test, and expressed by the I-square value. If substantial heterogeneity was observed (I-square value >50% and /or P<0.05), random-effects model was used to calculate pooled effect. Otherwise, a fixed-effects model was applied. Subgroup analysis was further conducted to identify the source of existed heterogeneity. HR values >1 indicated significant associations with poor prognosis. A sensitivity analysis was performed by removing each study at a time individually. The inverted funnel plots and Begg's tests were used to examine the publication bias. All of the P values were two-tailed and statistical significance was considered as a P<0.05. The meta-analysis was carried out by using Review Manager 5.20 and STATA software version 12.0.

3. Results

3.1. Summary of the included studies

As is shown in the flow chart (Figure 1), our initial

search identified 638 potentially relevant published literatures. After title and abstract review, 579 articles were removed according to the exclusion criteria. The remaining 59 articles were carefully analyzed and evaluated by reviewing the full texts. Of these, 10 articles were further excluded for molecular research, 13 studies were removed as secondary literature, 9 articles were overlapping date sets, 18 studies failed to provide key statistics for the extraction of HRs and 95%CI. Finally, nine articles are considered eligible to evaluate the prognostic role of miR-21 in NSCLC.

The main characteristics of the included eligible studies are summarized in Table 1. We collected date from 9 articles including a total of 1639 patients from China, Japan, Norway, and the United States. The participants were identified as either Caucasian or Asian based on their ethnic background. All of the research articles were about NSCLC. Six studies involved both squamous cell carcinoma and adenocarcinoma, two studies involved only adenocarcinoma[14,17] , and one studies involved square cell carcinoma[18]. MiR-21 expression was detected by quantitative real-time PCR (qRT-PCR) with seven in frozen tissue, two in Paraffin-embedded tissues[12,19], and two in serum[20,21]. In addition, the cut-off values of miR-21varied in each study, with median used in 6 studies and 5-fold or 2-fold applied in other studies. 10 studies mainly emphasized OS and one investigated RFS.

Table 1. Main characteristics of the eligible studies included in this meta-analysis

Study	Year	N	Ethnicity	Histology	Stage	Sample	Cut-off	Results
Saito	2011	191	Asian	AC	I II	Frozen tissues	Median	RFS
Saito	2011	126	Caucasian	AC	I II III	Frozen tissues	Median	OS
Voortman	2010	631	Caucasian	AC SC	I II III	Paraffin-embedded tissues	Median	OS
Gao	2010	47	Asian	AC SC	I II III	Frozen tissues	Median	OS
Gao	2011	30	Asian	SC	I II III	Frozen tissues	Median	OS
Wang	2011	88	Asian	AC SC	I II III	Serum	5-fold	OS
Markou	2001	48	Caucasian	AC SC	I II III IV	Frozen tissues	2-fold	OS
Liu	2012	70	Asian	AC SC	I II III IV	Serum	2-fold	OS
Liu	2012	70	Asian	AC SC	I II III IV	Frozen tissues	2-fold	OS
Tian	2015	204	Asian	NSCLC	I II III	Paraffin-embedded tissues	Median	OS
Robles	2016	204	Asian+ Caucasian	AC	I	Frozen tissues	Median	OS

AC, adenocarcinoma; SCC, squamous cell carcinoma; OS(overall survival); RFS (recurrence-free survival).

3.2. Correlation between miR-21 expression and NSCLC prognosis

For studies evaluating the correlation between miR-21 and NSCLC prognosis, we found that elevated miR-21 expression significantly associated with worse NSCLC survival (HR=2.29, 95%CI: 1.58-3.33, P<0.0001) (I^2=88%, P<0.00001) (Figure 2). 10 studies in all were subject to analysis miR-21 and OS, the pooled HR for OS was 2.26 (95%CI: 1.52-3.35, P<0.0001) (I^2=89%, P<0.00001) (Figure 3). Significant heterogeneity was found among these studies, so random-effect model was used. To reduce the influence of heterogeneity, subgroup analysis was performed to evaluate the effect of miR-21 expression. The analyzed subgroups were stratified based on the histology, type of specimen and ethnicity. In the histology subgroup analysis, over-expression of miR-21 reduced the OS of the patients with lung adenocarcinoma (HR=2.28, 95%CI: 1.51-3.42, P<0.0001), without apparent heterogeneity (I^2=0%, P=0.95) (Figure 4). In the sample subgroup analysis, higher miR-21expression was predictive of poorer OS both in frozen tissue sample (HR=2.33, 95%CI=1.42-3.83, P=0.0008) (I^2=75%, P=0.01) (Figure 5) and serum sample (HR=2.49, 95%CI=1.85-3.35, P<0.00001) (I^2=0%, P=0.32) (Figure 6). In the ethnicity subgroup analysis, elevated miR-21expression was more indicative of shortened OS in Asian NSCLC patients (HR=2.88, 95%CI: 1.64-5.08, P=0.0002) (Figure 7) than Caucasian cohorts (HR=1.57, 95%CI: 0.68-3.65, P=0.29) (Figure 8).

3.3. Publication bias and sensitivity analysis

Funnel plots and Egger's test were performed to assess the publication bias. As shown in Figure 9, the

funnel plots were nearly symmetric in included studies, the corresponding P values greater than 0.05 (Begg's P=0.164). Thus, there was no significant publication bias in the meta-analysis.

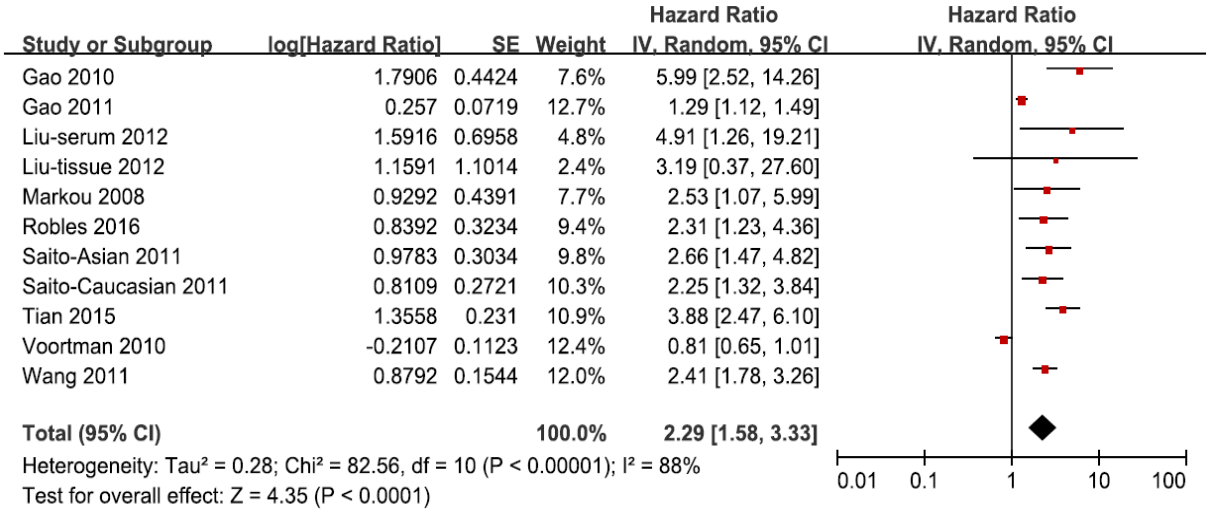

Figure 2. The relationship between miR-21 expression and non-small-cell lung cancer (NSCLC) prognosis.

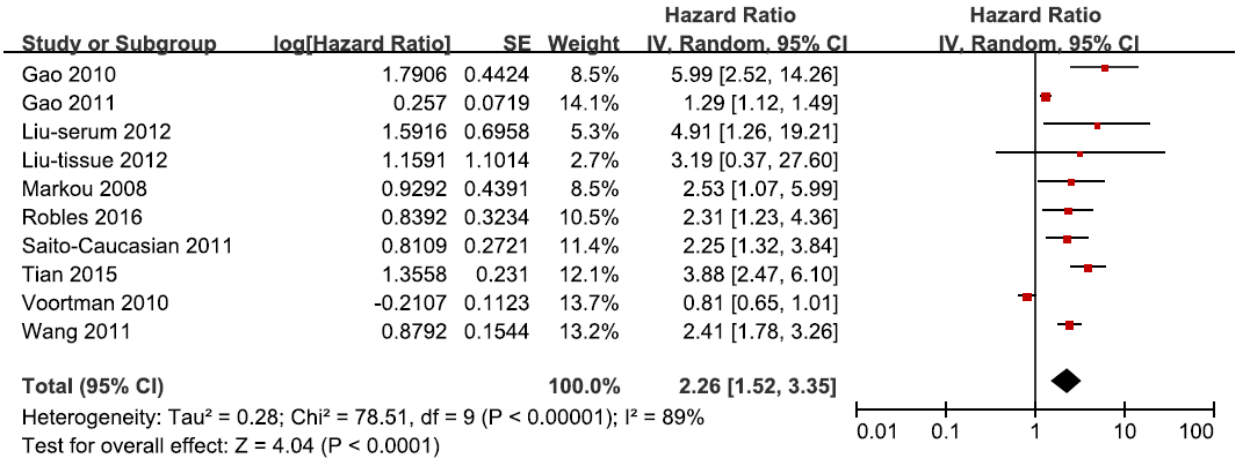

Figure 3. Forest plots of the analyses about miR-21 and overallsurvival(OS).

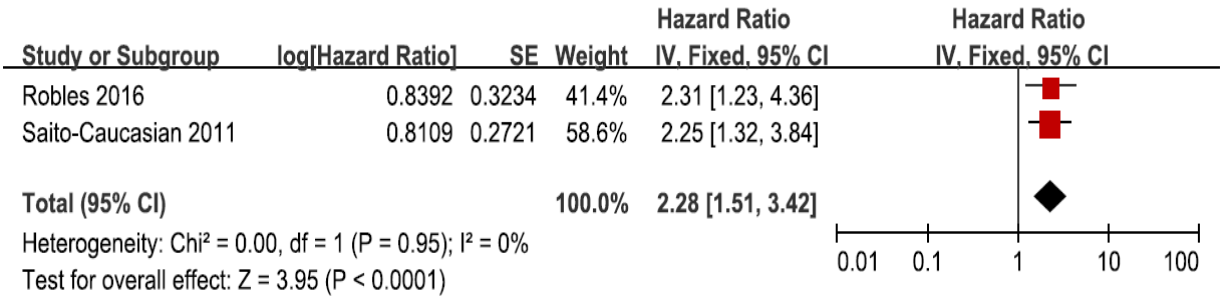

Figure 4. Forest plots of OS are reported as AC (adenocarcinoma).

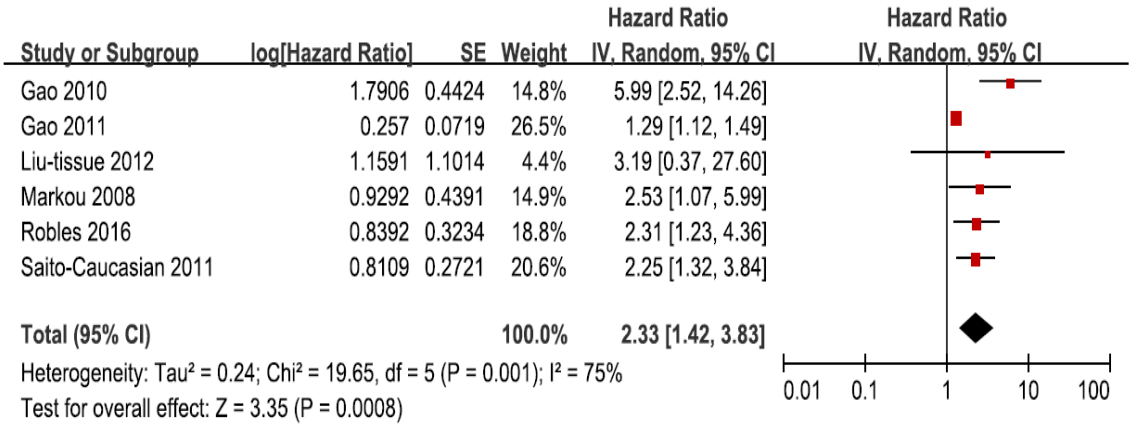

Figure 5. Forest plots of OS are reported as frozen tissue sample.

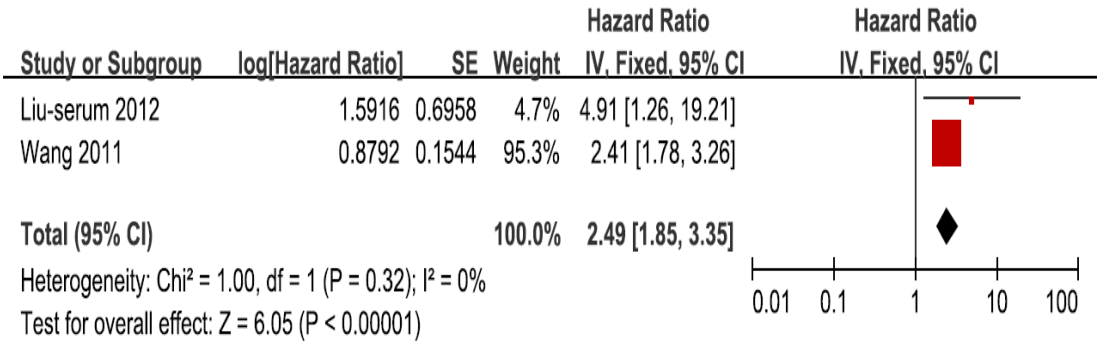

Figure 6. Forest plots of OS are reported as serum sample.

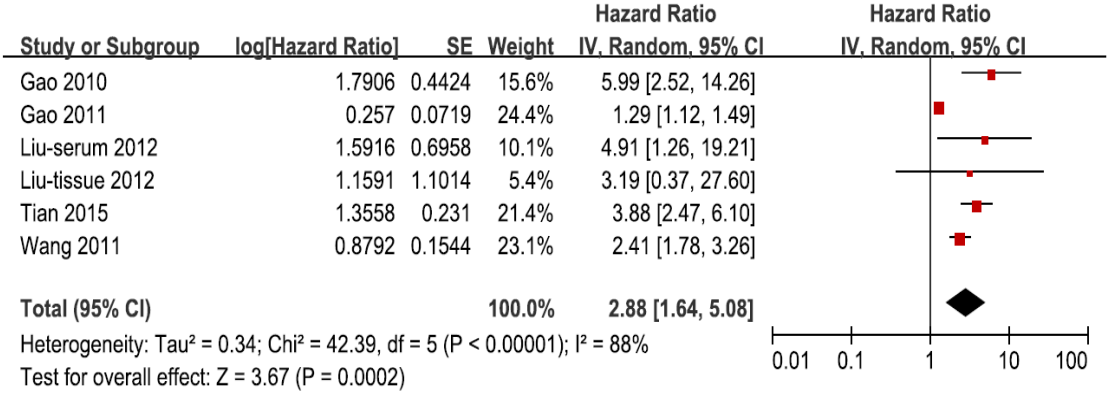

Figure 7. Forest plots of OS are reported from Asian.

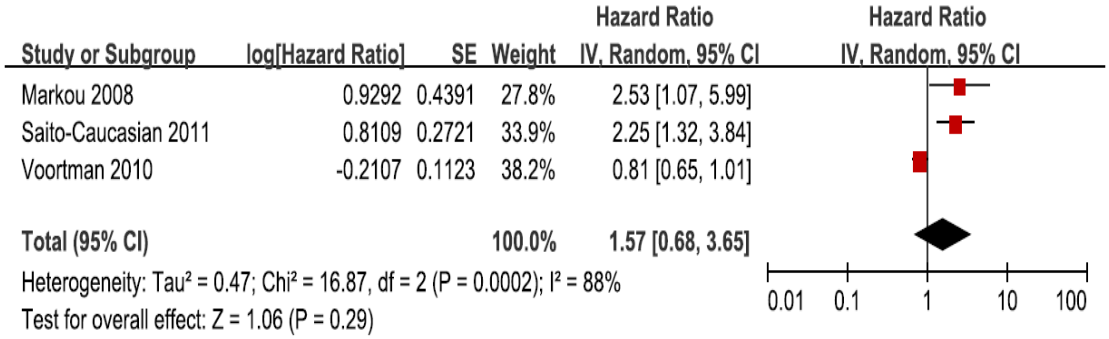

Figure 8. Forest plots of OS are reported from Caucasian.

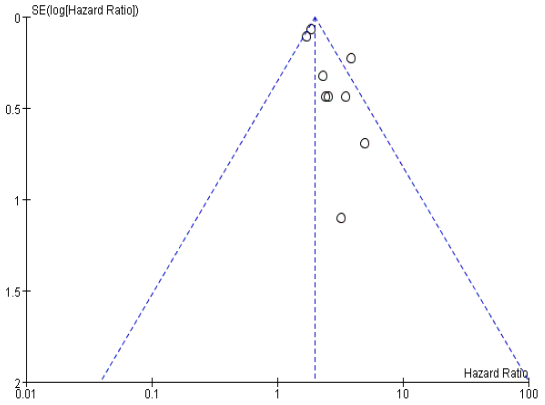

Figure 9. Funnel plots of studies included in the meta-analysis.

4. Discussion

Development of novel noninvasive prognostic biomarker is critical for predicting NSCLC patients' outcome and tailoring the clinical treatment. Recent efforts in cancer biology have revealed that microRNA (miRNA) could play a potential role as diagnostic and prognostic biomarkers of all kinds of malignant tumors. Among various miRNAs, miR-21 may be most attractive for clinical application because it was found aberrantly expressed in various carcinomas. Xia et al. reported that high-level miR-21 predicts unfavorable OS in colorectal cancer (HR=1.76 95%CI: 1.34-2.32, P<0.001)[22]. A meta-analysis by Xu et al. demonstrated that higher miR-21 expression also indicates worse overall and relapse-free survival in general carcinomas (HR=1.903, 95%CI: 1.713-2.113, P<0.001), including lung cancers and pancreatic carcinomas, moderately well on the basis of 25 studies[23] . However, controversy concerning the prognostic ability in NSCLC patients still exists in literature.

In the current meta-analysis, the pooled HR from 1639 patients in 9 studies for OS was 2.26 (95%CI: 1.52-3.35, P<0.0001), which was considered strongly predictive. Significant association was verified between promoted miR-21 level and poor NSCLC long-term survival. The results were consistent with the recently published meta-analysis conducted by Xu BH et. al[23]. However, we included newly published studies and performed subgroup analyses according to the type of sample. Nowadays, circulating miR-21 has been actively proposed as a high stability and non-invasive biomarker of prognosis for cancers. In the sample subgroup analysis, our meta-analysis revealed that circulating miR-21 level may be an effective prognostic indicator for NSCLC. Notably, the results of the ethnicity subgroup analysis showed that over-expression of miR-21 significantly predicted shorter OS in Asians, whereas the prediction was not significant in Caucasians.

Nonetheless, several limitations exist in our meta-analysis. First, the substantial heterogeneity among the eligible articles is caused by the differences in the baseline characteristics of participants (tumor stage, age, country, or race), type of specimen, detection methods and the cut-off values of miR-21. In order to minimize the effect of these differences, a random-effect model was used. Simultaneously, we performed subgroup analysis based on type of specimen, histology and ethnic background. Heterogeneity eliminated significantly in the serum sample and lung adenocarcinoma group but not in Asian group. Second, the small number of eligible studies and potential publication bias may have affected the results of the analysis. Additionally, due to the lack of uniform cut-off value, we failed to specifically define miR-21 over-expression in this meta-analysis.

In conclusion, the present meta-analysis has demonstrated that over-expression of miR-21 can predict unfavorable NSCLC prognosis, especially in Asians. Meanwhile, we found that serum miR-21 level was also significantly associated with poor survival in NSCLC patients. However, more large-scale and standard studies are warranted to confirm the role of miR-21 in lung cancer prognosis and clinical application in future.

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