

A meta-analysis on the relationship of exosomes and the prognosis of lung cancer

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Abstract

Background: A lot of research evidence shows that exosomes play an indelible role in the prognosis of lung cancer, but there are many disputes. Therefore, we conduct a meta-analysis to further demonstrate.

Methods: A literature retrieval was performed through a search of PubMed, Embase, Web of Science, Cochrane, CKNI, Wanfang, and other databases to locate documents from the literature that satisfied the inclusion criteria. There were four outcome indicators: overall survival (OS), disease-free survival (DFS), disease-specific survival (DSS), and progression-free survival (PFS). Subgroup analysis was conducted according to sample size, country, detection method, analysis method, and pathological type. Stata 14.0 software was used to evaluate the prognostic value of exosomes in lung cancer.

Results: A total of 2456 patients with lung cancer from 29 studies in 16 articles were included. The expression level of exosomes was closely associated with the OS and DFS of patients, although no statistical difference was observed between exosomes and DSS or PFS. Eighteen studies with 2,110 patients were evaluated to examine the prognostic value of exosomes in lung cancer by exploring the association between exosomes and OS. The results showed that exosomes were strongly associated with worse OS, and the combined hazard ratio (HR) was 2.01 (95% confidence interval [CI]: 1.70–2.39, $P = .000$). Six studies investigated the association between exosomes and DFS, and showed a pooled HR of 2.48 (95% CI: 1.75–3.53, $P = .000$).

Conclusion: Our analysis indicated that the expression level of exosomes was closely associated with the OS and DFS of patients with lung cancer, suggesting that exosomes are associated with poor prognosis of lung cancer. Exosomes may be a new biomarker for the prognosis of lung cancer, although a large number of prospective studies are still needed to support this.

Abbreviations: ADC = adenocarcinoma, DFS = disease-free survival, DSS = disease-specific survival, HR = hazard ratio, LCC = Large cell carcinoma, NSCLC = non-small cell lung cancer, OS = overall survival, PFS = progression free survival, SCLC = small cell lung cancer.

Keywords: exosomes, lung cancer, meta-analysis, prognosis

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1. Introduction

Lung cancer is one of the main causes of cancer death in the world, ranking first in terms of morbidity and prevalence.^[1] Recently, considerable progress has been achieved in the diagnosis and treatment of lung cancer, although the 5-year survival rate is still very low, at only about 15%.^[2,3] Finding biomarkers for more sensitive diagnosis or prognostic monitoring remains an ongoing task and goal for the medical field. At present, blood-based biomarkers are receiving more and more attention in the diagnosis and prognosis of cancer.

Exosomes play an important role in the transmission of substances and information in the occurrence and development of tumors, which can affect their progression.^[4,5] Therefore, the bioactive substances carried by exosomes may be used as biomarkers for diagnosis and prognosis of tumors.^[6] Exosomes are produced by a number of cell types and are mainly secreted by hematopoietic cells, such as reticulocytes, B lymphocytes, T lymphocytes, and dendritic cells, through a series of processes, such as “endocytosis-fusion-exclusion”.^[7–9] Exosomes are also produced by cells of non-hematopoietic origin, like epithelial cells (intestinal epithelial cells), neurons, fibroblasts, and tumor cells.^[7–9] Exosomes are membrane-encapsulated extracellular small vesicles, with a diameter of about 30–100 nm.^[7] They have a lipid bilayer membrane structure,^[10] and their vesicles also contain a large amount of bioactive substances, including

proteins, RNA, DNA and lipids.^[11,12] Exosomes can be separated from various fluids, including plasma, serum, urine, saliva, nasal secretions, bronchoalveolar lavage, pleural effusion, bile, ascites, semen, breast milk, amniotic fluid, and cerebrospinal fluid.^[13–17] They can also be stored very stably at temperatures as low as -20°C .^[10] Exosome membranes are mainly composed of lipids, which make them highly stable and easily absorbed by target cells.^[18] These characteristics make exosomes useful as biomarkers.

With the development of proteomics and genomics, there is an increasingly profound understanding of the source, structure, and function of exosomes. In recent years, research on exosomes and lung cancer prognosis has become a medical hotspot. Majority of studies have found that exosomes play a vital role in the diagnosis, treatment, and prognosis of lung cancer, although the results remain controversial. This study aimed to assess the association between exosomes and the prognosis of lung cancer through meta-analysis.

2. Materials and methods

2.1. Document retrieval

Studies were identified via an electronic search of PubMed, Embase, Web of Science, Cochrane, CKNI, and Wanfang. Data up to February of 2020 were included. Search terms included: exosome OR exosomes, lung cancer OR lung carcinoma OR lung neoplasm, OS OR overall survival OR mortality OR survival, prognostic value, PFS OR progression-free survival, and DFS OR disease-free survival. The retrieval languages were Chinese and English. All analyses were based on previous published studies, thus no ethical approval and patient consent are required.

2.2. Literature inclusion and exclusion criteria

Inclusion criteria were

1. publicly published studies on the association between exosomes and the prognosis of lung cancer;
2. patients were diagnosed with lung cancer by pathology or cytological examination;
3. outcome indicators of overall survival (OS), disease-free survival (DFS), disease-specific survival (DSS), and progression-free survival (PFS), and
4. ability to extract hazard ratio (HR) and 95% confidence intervals (CIs) from published Chinese and English literature directly or indirectly.

Exclusion criteria were:

1. documents that did not comply with the inclusion criteria;
2. documents that could not provide complete information or data;
3. literature such as reviews, case reports, and animal or cell experiments; and
4. repeated publications.

2.3. Data extraction and quality evaluation

Two researchers independently screened the recovered documents according to the inclusion and exclusion criteria of the literature. If there were disagreements, a third researcher was consulted to provide assistance in resolution. The researchers

assessed the quality of the literature that met the requirements and extracted data from them, including author, nationality of the research subjects, publication time, sample size, and detection method. The Newcastle-Ottawa Scale (NOS) score was used to evaluate the quality of the included studies, with 9 points possible. Studies with scores of ≥ 6 points were included.^[19]

2.4. Statistical analyses

Stata 14.0 was used for analysis. Subgroup analysis was performed based on sample size, country, detection method, analysis method, and pathological type to evaluate the potential sources of heterogeneity. Outcome indicators were survival rate, survival curve, and hazard ratio (HR). If the Kaplan-Meier survival curve was provided in the literature, the 5-year survival rate was obtained using Engauge Digitizer 1.4, and the HR was obtained using RevMan 5.3.^[20–22] Heterogeneity analyses used the I^2 and Q tests. When $I^2 < 50\%$ and the Q test $P > .1$, it was presumed that there was no heterogeneity, and the fixed effect model was used for analysis; otherwise, the random effect model was selected. If heterogeneity was present, sensitivity analysis was used by eliminating a single study in a queue to identify the potential sources of heterogeneity and assess whether they would affect the stability of the overall results. The main source of heterogeneity was analyzed by meta-regression. Funnel charts and Begg and Egger tests were used to detect publication bias. The trim-and-fill method was used to further analyze the impact of publication bias.

3. Results

3.1. Literature screening results

A total of 235 articles were initially screened, and 190 articles remained after 45 duplicates were eliminated. The literature was then screened according to title and abstract, and 153 more studies were excluded. The full text of the remaining 37 studies was read closely, and 21 additional studies were excluded for the following reasons: 14 studies lacked complete data, and 7 studies had no data available. Finally, the remaining 16 articles met the requirements and were included in this study (Fig. 1).^[23–38]

3.2. Basic characteristics and quality evaluation of literature

Our study included 16 articles that included 29 studies, with 18 studies on OS, six on DFS, two on DSS, and three on PFS. Because of the different types of exosomes mentioned in the articles, it was possible there were multiple studies in the same article, although quality evaluations were conducted to ensure the inclusion criteria were met. Of the 18 studies on exosomes and OS, 15 were from China, and the rest were from Japan and Europe. There were 11 studies with sample sizes > 100 , and there were 15 that used qRT-PCR as a measure of exosome expression. There were eight studies that used univariate regression analysis; the others used multivariate regression analysis. The pathological types of cases included in the 18 OS studies were: non-small cell lung cancer (NSCLC), adenocarcinoma (ADC), and small cell lung cancer (SCLC). Among them, nine studies were about NSCLC, eight were about ADC, and one was about SCLC. Of the nine studies on NSCLC, Xiong^[25] included ADC (42 cases) and SQCC (58 cases); Liu^[37] included ADC (115 cases), SQCC (73

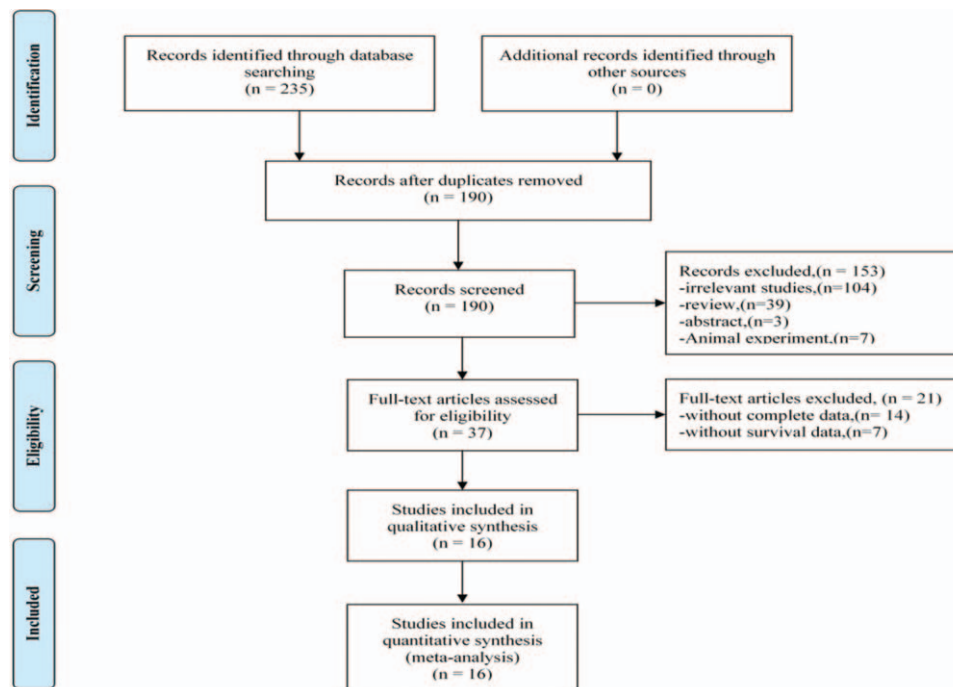


Figure 1. Flow diagram showing study retrieval and selection process.

cases), large cell carcinoma (LCC) (3 cases), and others (1 case); Dejima^[36] included ADC (134 cases), SQCC (53 cases), and others (one case); Paulsen^[38] included ADC (198 cases), SQCC (69 cases), and others (nine cases); Xu^[28] included ADC (12

cases) and SQCC (31 cases); Zhang^[2,3] included ADC (53 cases), SQCC (46 cases), and others (4 cases); and Kanao^[34] included ADC (201 cases) and SQCC (72 cases). The specific number of lung cancer subtypes could not be obtained in Yuwen^[30] or

Table 1

Basic characteristics of included studies.

Author	Year	Country	Age	Exosome type	Dysregulation	Sample (n)	Pathological type	Analysis method	Detection method	Oncologic outcomes	NOS
Zhang	2020	China	57.4	miR-378	Upregulation	103	NSCLC	MVA	qRT-PCR	OS	8
Xue	2020	China	NA	miR-151a-5p	Upregulation	6	ADC	UVA	qRT-PCR	OS	7
				miR-10b-5p	Upregulation	6	ADC	UVA	qRT-PCR	OS	
				miR-192-5p	Upregulation	6	ADC	UVA	qRT-PCR	OS	
				miR-106b-3p	Upregulation	6	ADC	UVA	qRT-PCR	OS	
				miR-484	Upregulation	6	ADC	UVA	qRT-PCR	OS	
Xiong	2020	China	NA	miR-214	Upregulation	100	NSCLC	UVA	qRT-PCR	OS	6
Shimada	2020	Japan	NA	UCHL1	Upregulation	72	SCLC	MVA	qRT-PCR	DFS	7
Nanou	2020	Netherland	65	tDEVs	Upregulation	137	NSCLC	UVA	Cell search	OS	8
Xu	2019	China	64	miR-32	Downregulation	43	NSCLC	UVA	qRT-PCR	OS/PFS	6
Sun	2019	China	NA	miR-423-3p	Upregulation	155	ADC	MVA	qRT-PCR	OS	7
				miR-4270	Downregulation	155	ADC	MVA	qRT-PCR	OS	
Yunwen	2018	China	NA	miR-425-3p	Upregulation	170	NSCLC	UVA	qRT-PCR	PFS	8
Li	2018	China	NA	FECRS	Downregulation	56	SCLC	UVA	qRT-PCR	PFS	7
Koh	2018	Korea	66	Rab27B	Upregulation	96	SQCC/ADC	MVA	IHC	DFS/DSS	6
				Rab27B	Upregulation	37	SQCC/ADC	MVA	IHC	DFS/DSS	
Xu	2018	China	60	miR-21	Upregulation	437	ADC	UVA	qRT-PCR	OS	7
Kanaoka	2018	Japan	NA	miR-451a	Upregulation	285	NSCLC	MVA	qRT-PCR	OS/DFS	9
Zeng	2017	China	55	LncRNA	Upregulation	86	SCLC	MVA	qRT-PCR	OS	7
				miR-21	Upregulation	201	NSCLC	MVA	qRT-PCR	DFS	7
Dejima	2017	Japan	NA	miR-4257	Upregulation	201	NSCLC	MVA	qRT-PCR	DFS	
				miR-23b-3p	Upregulation	196	NSCLC	MVA	qRT-PCR	OS	8
				miR-10b-5p	Upregulation	196	NSCLC	MVA	qRT-PCR	OS	
Paulsen-S	2016	Danmark	68.6	miR-21-5p	Upregulation	196	NSCLC	MVA	qRT-PCR	OS	
				Alix	Upregulation	276	NSCLC	MVA	Spotbot	OS	8

ADC = adenocarcinoma, DFS = disease-free survival, DSS = disease-specific survival, IHC = immunochemistry, MVA = multivariate analysis, NA = not available, NOS = Newcastle-Ottawa scale, NSCLC = non small cell lung cancer, OS = overall survival, PFS = progression-free survival, qRT-PCR = quantitative real-time PCR, SCLC = small cell lung cancer, SQCC = squamous cell carcinoma, UVA = univariate analysis.

Nanou.^[27] The included documents were evaluated using the NOS score, with a total possible score of 9 points, and scores of ≥ 6 points indicated high-quality studies, as shown in Table 1.

3.3. Meta-analysis results

There were 18 studies in the OS group, and there was heterogeneity among the studies ($I^2=67.8\%$, $P = .000$), therefore, the random effects model was used. The results showed that the HR between exosomes and the OS of patients with lung cancer was 2.01 (95% CI: 1.70–2.39, $P = .000$) (Fig. 2). The DFS group contained six studies, and no obvious heterogeneity ($I^2=30.9\%$, $P = .204$) was found among them. Therefore, the fixed effect model was selected. The results indicated that the combined HR between exosomes and DFS of patients with lung cancer was 2.48 (95% CI: 1.75–3.53, $P = .000$) (Fig. 3). In the OS group, patients with abnormal exosome expression had a 101% higher risk of poor prognosis (HR=2.01, 95% CI: 1.70–2.39, $P = .000$), and for the DFS group, patients with abnormal exosome expression had a 148% higher risk of poor prognosis (HR=2.48, 95% CI: 1.75–3.53, $P = .000$). The DSS and PFS groups included two and three studies, respectively. The results showed that there was no

statistical significance between exosomes and DSS or PFS ($P = .356 > 0.05$, $P = .058 > .05$) (Fig. 4).

3.4. Subgroup analysis

To further clarify the source of heterogeneity in the OS group, we conducted a subgroup analysis based on the sample size, country, detection method, analysis method, and pathological type. The results showed that the expression level of exosomes was significantly associated with the OS, as shown in Table 2. After the subgroup analysis, no obvious source of heterogeneity was found, therefore, further meta-regression and sensitivity analyses were required.

3.5. Sensitivity analysis and meta-regression

Sensitivity analysis was used to evaluate the stability of the results by deleting each study in a queue and then recombining the HR for OS. We observed that the heterogeneity of the combined HR for OS was mainly caused by one study^[29]; the combined HR after its exclusion was 1.95 (95% CI: 1.68–2.26, $P = .008$). However, there was no significant change before and after the exclusion, indicating that the combined HR was robust (Fig. 5).

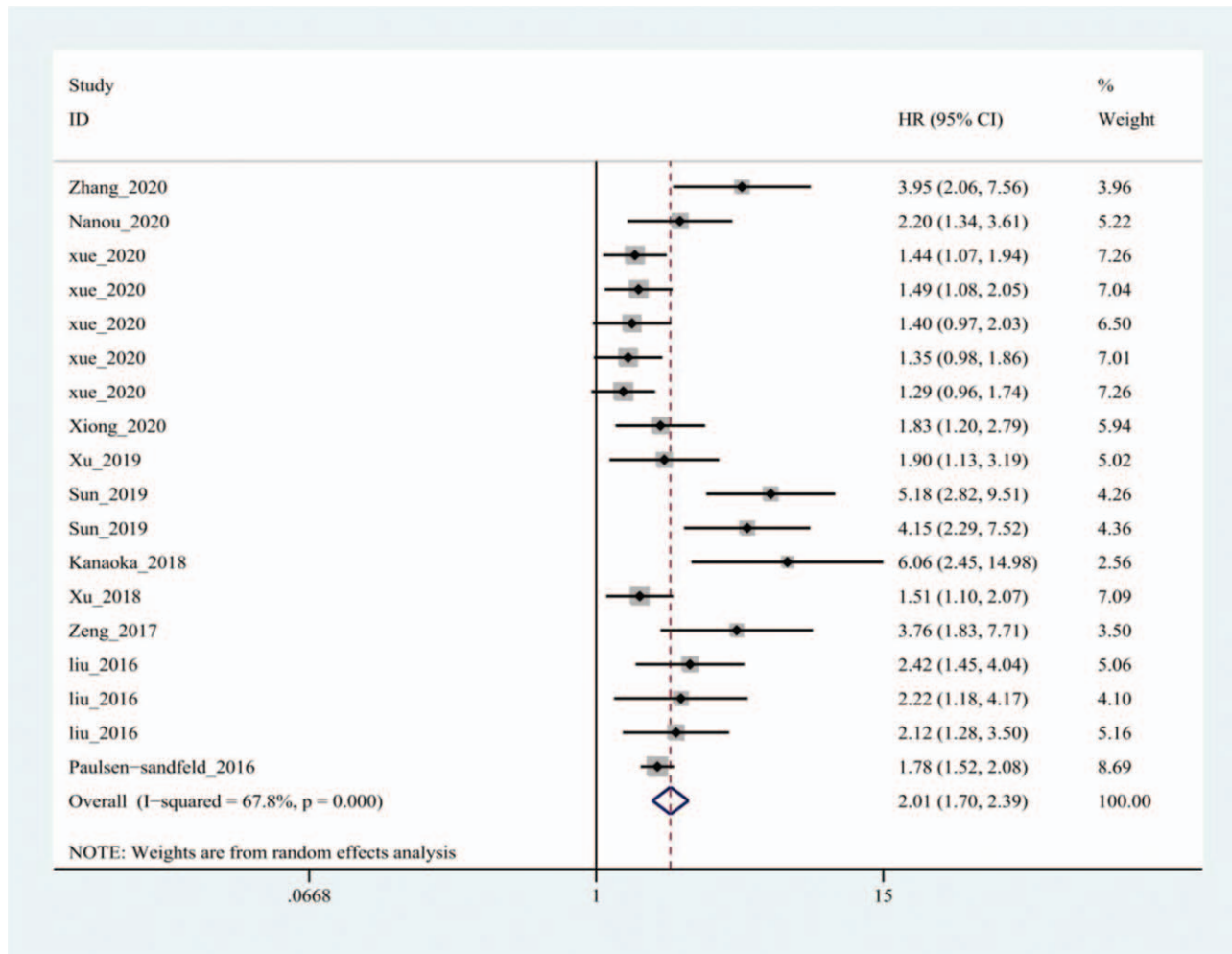


Figure 2. Forest plot of the Hazard ratio for the relationship between exosomes and overall survival of lung cancer patients.

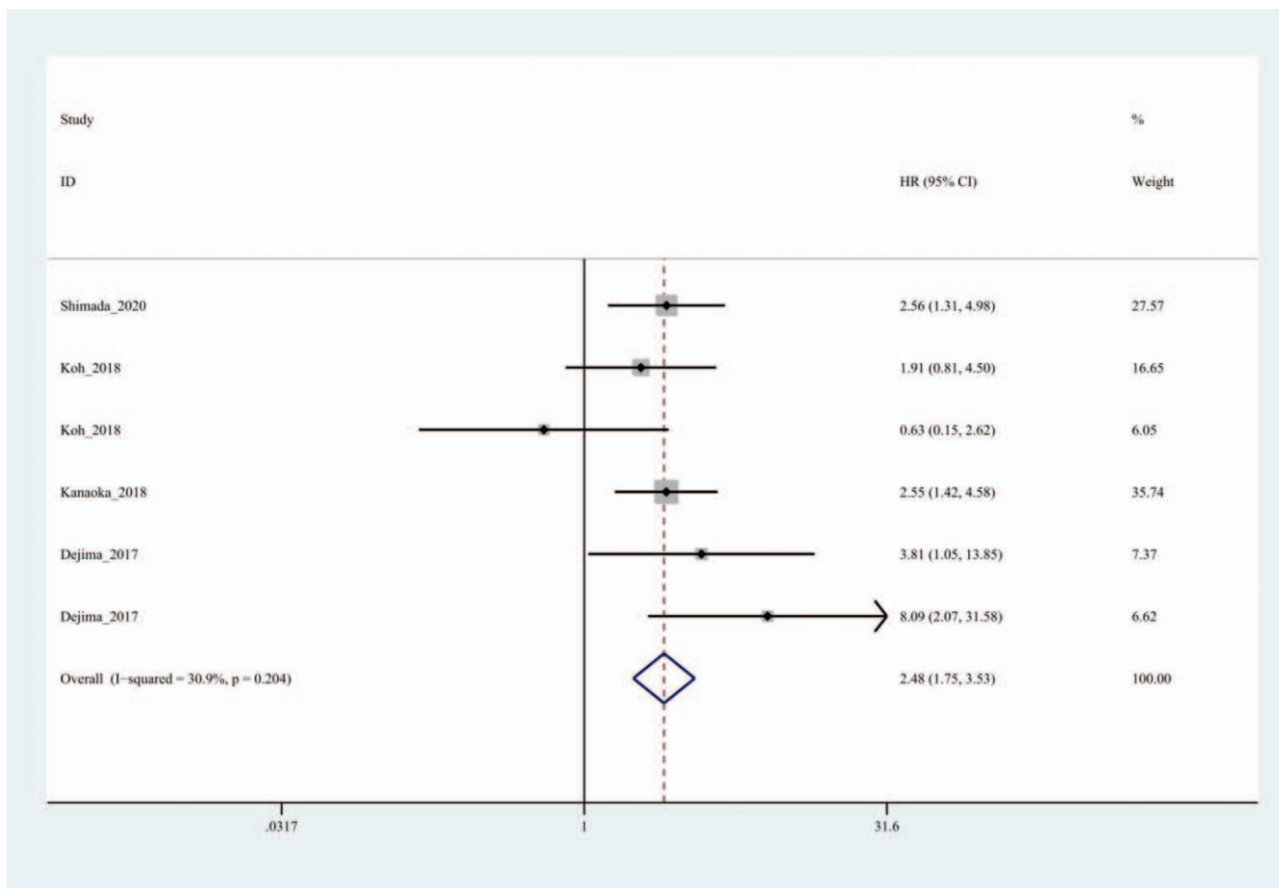


Figure 3. Forest plot of the Hazard ratio for the relationship between exosomes and disease-free survival of lung cancer patients.

The results of the meta-analysis are shown in Table 3, which shows that the analysis method ($P = .001$) may be the main source of heterogeneity, and other factors, such as sample size ($P = .934$), country ($P = .506$), and detection method ($P = .63$), have no statistical significance.

3.6. Publication bias

3.6.1. Funnel graph. We detected publication bias using funnel plots in the OS and DFS groups. The funnel plots showed that the OS group was asymmetrical, suggesting that there might be publication bias, while in the DFS group, the funnel plot showed

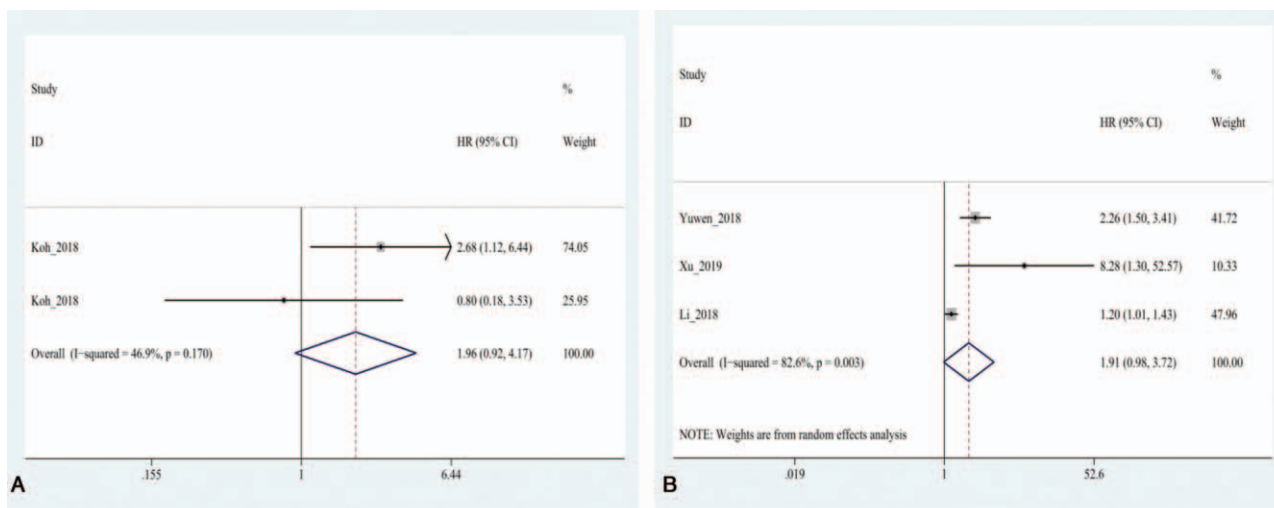


Figure 4. Forest plot of the Hazard ratio for the relationship of exosomes and the prognosis of lung cancer patients. (A) The overall pooled HR for disease-specific survival of lung cancer patients. (B) The overall pooled HR for progression free survival of lung cancer patients.

Table 2.
Summary of overall and subgroup analyses for exosomes on OS.

	Studies (n)	Combined HR (95%CI)	Weight (%)	I ²	P-value (Q)
overall	18	2.01 (1.70-2.39)	100.0	67.8%	.000
Country					
China	15	1.98 (1.62-2.42)	83.53	68.7%	.000
Other countries	3	2.43 (1.45-4.08)	16.47	72.5%	.026
Sample size					
>100	11	2.48 (1.95-3.15)	56.41	68.1%	.001
<100	7	1.50 (1.27-1.77)	43.59	30.2%	.198
Detection method					
qRT-PCR	15	2.08 (1.69-2.55)	86.09	71.2%	.000
Non qRT-PCR	3	1.82 (1.56-2.11)	13.91	0.0%	.424
Analysis method					
MVA	10	2.90 (2.17-3.86)	46.88	69.1%	.001
UVA	8	1.46 (1.30-1.65)	53.12	0.0%	.877
Pathological type					
NSCLC	9	2.19 (1.80-2.67)	45.72	38.9%	.109
ADC	8	2.01 (1.70-2.39)	50.78	75.4%	.000
SCLC	1	3.76 (1.83-7.71)	3.50	–	–

ADC=adenocarcinoma, DFS=disease-free survival, DSS=disease-specific survival, MVA=multivariate analysis, NSCLC=non small cell lung cancer, OS=overall survival, PFS=progression-free survival, qRT-PCR=quantitative Real-time PCR, SCLC=small cell lung cancer, UVA=univariate analysis.

symmetry, which indicated no obvious publication bias, as shown in Figure 6. The bias in the OS group needed to be further verified using the Begg’s and Egger’s tests.

3.6.2. Begg’s and Egger’s test. The Begg’s and Egger’s tests were performed to assess the publication bias of the OS group (Fig. 7). These tests suggested that there was a publication bias ($P=.000<.05$, $P=.000<.05$). Therefore, we needed to employ the trim and fill method to further evaluate the stability of the combined HR for OS.

3.6.3. Trim and fill method. The final result is shown in Fig. 8. The adjusted funnel plots for OS became symmetrical, and the combined HR (HR=1.64, 95% CI: 1.35-1.98) for OS only changed marginally after the trim and fill method was applied, indicating the stability and reliability of our analysis.

4. Discussion

It is usually difficult to predict the prognosis of lung cancer. It is well known that tumor size, clinical stage, pathological grading,

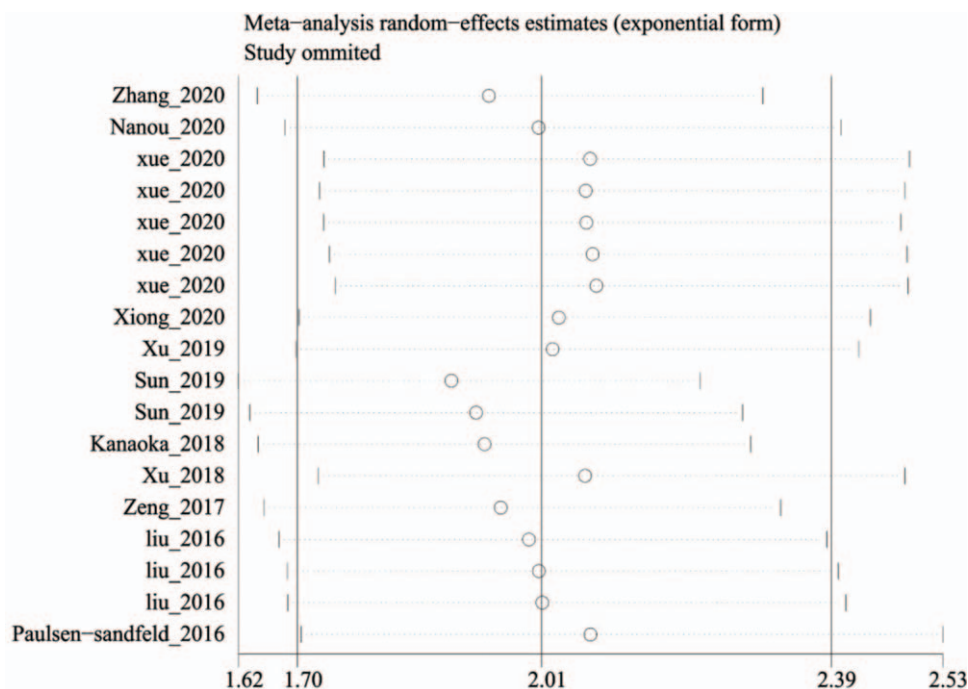


Figure 5. Sensitivity analyses to assess the effect of individual studies on the overall pooled HR for overall survival of lung cancer patients.

Table 3
Meta-regression analysis of potential source of heterogeneity.

Heterogeneity factors	OR	SE	t	P-value	95%CI
Sample size	1.087	0.149	0.61	.934	0.81–1.46
Country	0.512	0.244	−1.41	.506	0.18–1.43
Analysis method	1.988	0.316	4.32	.001	1.41–2.80
Detection method	3.338	1.562	2.58	.063	1.21–9.18

OR=odds ratio, SE=standard error.

classification, and other characteristics are the main factors that determine the prognosis of lung cancer, although we still need more and simpler indicators. Like most cancers, lung cancer is inclined to recur, especially in the course of treatment. We hope that these new indicators can reflect their short-term and long-term prognoses to better guide clinical work. A large amount of evidence shows that numerous genetic markers affect the biological behavior of lung cancer, although the inherent nature of gene disorders that lead to cancer recurrence, progression, or metastasis remains elusive.^[39] With these factors in mind, it is essential to study the material characteristics associated with the biological behavior of cancer.

Tumor-secreted factors play a guiding role in the local and far-reaching effects of tumors, such as soluble factors and exosome nanovesicles.^[40] Exosomes have potent biological functions. They play an important role in mediating tumor immune escape in many cancers, such as gastric cancer, liver cancer, and lung cancer, thereby creating conditions for tumor cells to grow *in vivo*.^[41] They can also cause tumorigenesis by promoting the transformation of cell epithelium and stroma and cell proliferation. Further, they can affect the invasion and metastasis of cancer by regulating cell apoptosis and the formation of the microenvironment before tumor metastasis.^[42–47] A majority of studies have shown that exosomes can induce angiogenesis in different tumors by regulating cytokines and growth factor

receptors, thus promoting the occurrence and development of tumors.^[48–51] Exosomes, as carriers of information, are inextricably associated with the occurrence and development of tumors. They are some of the most important potential biomarkers in cancer, and are worthy of in-depth exploration and research.

Our analysis of the included 16 articles that described 2456 cases showed that the expression level of exosomes was associated with the prognosis of lung cancer. These results suggest that expression of exosomes may play a vital role in the occurrence and progression of lung cancer, and that they may be a potential biological indicator of its prognosis.

Further subgroup analysis of the OS group indicated that the expression level of exosomes was significantly correlated with the OS of patients with lung cancer. There was no significant difference between the expression level of exosomes and the detection methods. At the same time, we found that most studies used qRT-PCR to detect exosomes, and the combined HRs were higher than those of the non-qRT-PCR group (HR=2.08, 95% CI: 1.69–2.55, HR=1.82, 95% CI: 1.56–2.11). There was no obvious difference between them, therefore, all HRs could be used for the detection of exosomes. The comparison between them needs to be further analyzed, however. Through subgroup analysis, it was found that the effect of abnormal exosome expression on the prognosis of lung cancer remains different. In

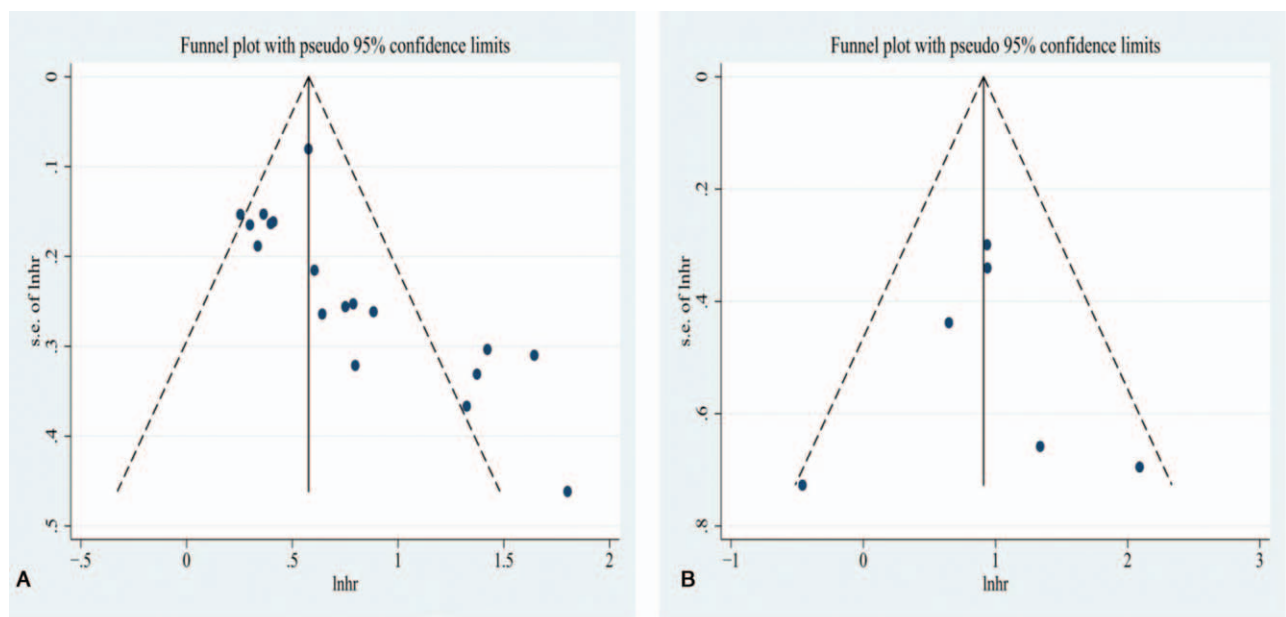


Figure 6. Funnel plots of the relationship between exosomes and the prognosis of lung cancer. (A) Relationship of exosomes and overall survival of lung cancer patients. (B) Relationship of exosomes and disease-free survival of lung cancer patients.

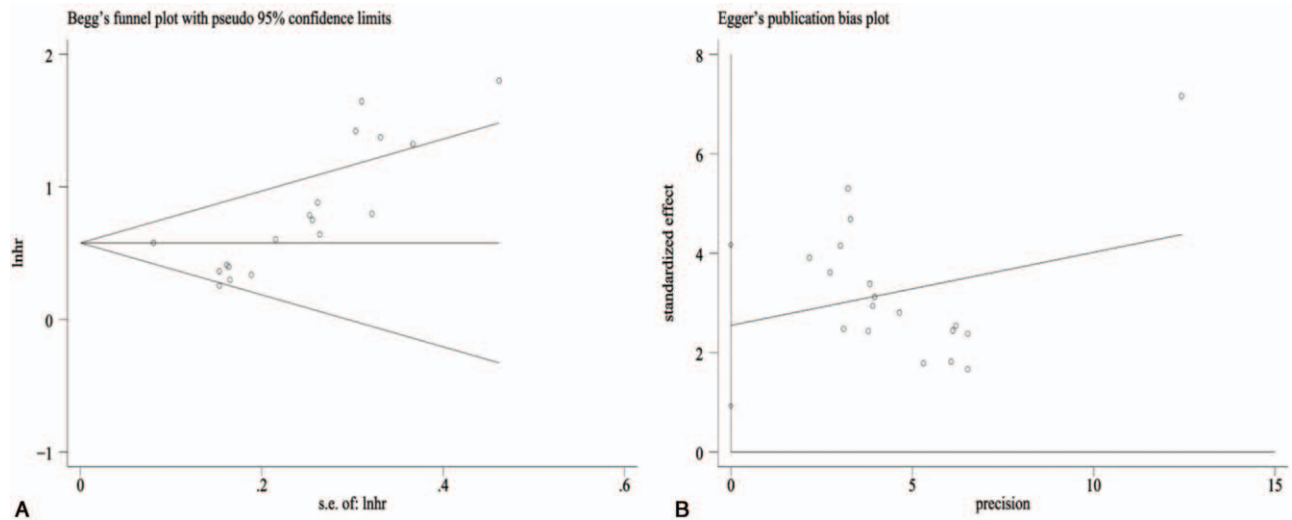


Figure 7. Funnel plots for publication bias of the relationship between exosomes and overall survival of lung cancer patients. (A) Begg's Test. (B) Egger's Test.

the multivariate analysis, the combined HRs were significantly higher than those in the univariate analysis (HR=2.90, 95% CI: 2.17–3.86, HR=1.46, 95% CI: 1.30–1.65). Our results also indicated that the expression level of exosomes was not only associated with the prognosis of lung cancer, but also might be linked to tumor stage, size, and metastasis. However, it is impossible to make a more accurate judgment using the literature alone, and a large amount of detailed research data is needed to further demonstrate these results. We also found regional differences between exosomes and patients' OS; for example, the combined HRs of studies from China were lower than those from other regions (HR=1.98, 95% CI: 1.62–2.42, HR=2.43, 95% CI: 1.45–4.08). However, due to the small number of studies from other countries, more research is needed to confirm these regional differences. In addition, subgroup analysis indicated that the expression of exosomes was different among the OS of patients with different pathological types of lung cancer

(HR=2.19, 95% CI: 1.80–2.67, HR=2.01, 95% CI: 1.70–2.39, HR=3.67, 95% CI: 1.83–7.71). In seven of the nine studies on NSCLC, out of a total of 1198 patients with NSCLC, 754 (63.78%) had ADC, 405 (33.81%) had SQCC, and nine (2.41%) had other cancers. The other two studies included 170 cases and 137 cases of NSCLC. No specific subtypes of lung cancer have been described in the reviewed literature. In addition, none of these seven studies counted the survival data of specific subtypes of lung cancer, which makes it impossible for us to further analyze the association between exosomes and pathological types. In the OS group, there was one study alone on exosomes and SCLC, therefore, whether there is a difference in pathological types needs to be verified by larger samples and multi-center data. Interestingly, many of the included studies are about miRNA, which suggest that miRNA may become a hotspot in exosome research in future studies. Furthermore, we performed meta-regression, which indicated that the analysis methods may be the

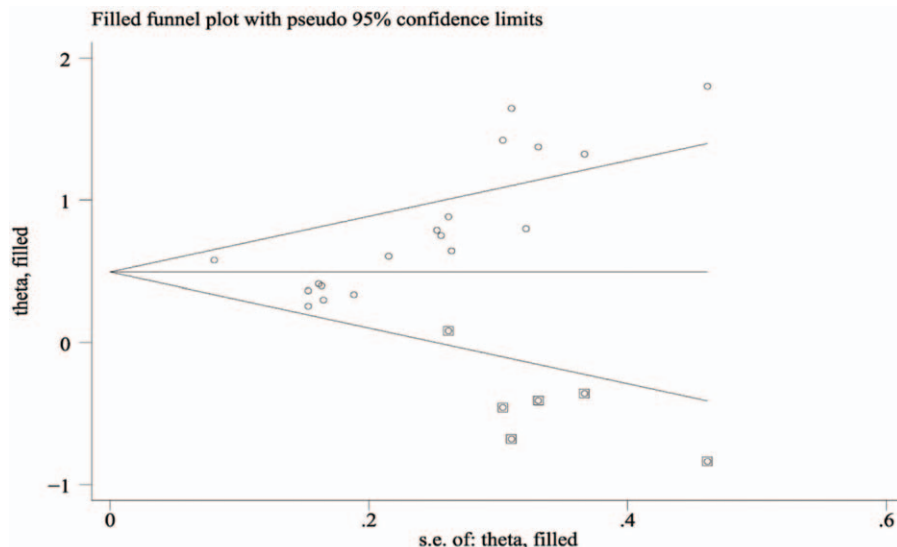


Figure 8. Adjusted Begg's funnel plot from the trim and fill method regarding the publication bias for overall survival.

main source of heterogeneity. Our study used sensitivity analysis to verify the impact of a single study on our analysis results, and the results indicated that our conclusion was relatively stable. At the same time, the included studies had publication bias, although the results of the trim and fill method nonetheless suggested that our analysis results were stable and reliable.

This meta-analysis has the following advantages:

1. strict inclusion criteria were adopted, and the overall NOS score was generally high; and
2. sensitivity analysis, meta-regression, and the trim and fill method were used to further evaluate the robustness of the meta-analysis results.

However, there are still some shortcomings in this study:

1. some studies lacked clinical parameters, such as different subtypes of lung cancer, degree of tumor differentiation, depth of invasion, TNM stage, etc., so that we could not further analyze the association between exosomes and lung cancer from these aspects;
2. because there are too few studies on the PFS and DSS groups, we cannot explain their associations with the expression levels of exosomes.
3. the study only included publications in the Chinese and English literature, which affected the comprehensiveness of the data; and
4. most cases included in the literature came from China, which might have resulted in regional bias.

5. Conclusions

In summary, the expression level of exosomes is closely associated with the poor prognosis of lung cancer. Our results have strong statistical significance, especially for the OS and DFS of patients with lung cancer. In the future, we will look forward to more studies on exosomes and PFS or DSS in lung cancer to more accurately explain the association. Therefore, exosomes can serve as prognostic markers for lung cancer. Our study also has good clinical application prospects. We should combine exosomes with additional lung cancer markers to jointly manage the prognosis of lung cancer.

Author contributions

Conception: Jingying Luo.

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Writing—review & editing: Hui Xiang, Miao Luo, Jingying Luo.

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