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Research article

Keywords: MicroRNA let-7, Non-small cell lung cancer, Prognosis, Overall survival, Meta-analysis

Posted Date: July 28th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-46072/v1>

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Prognostic Role of MicroRNA Let-7 in Non-Small Cell Lung Cancer: A Systematic Review and Meta-Analysis

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Abstract

Background Recent studies have shown that MicroRNAs can be used as potential biomarkers to the prognosis in non-small cell lung cancer (NSCLC). Hence, we conducted this meta-analysis to evaluate the prognostic role of microRNA let-7 in NSCLC patients.

Methods PubMed, Web of Science, Medline and the Cochrane Library were searched for relevant studies. Eligible studies were met specific inclusion and exclusion criteria. Stata 15.1 was used to calculate the pooled parameters.

Results A total of 11 studies involving 2278 NSCLC patients were included in this meta-analysis. Subgroup analyses established that the heterogeneity was connected with ethnicity and follow-up time. The results suggested that the low expression of microRNA let-7 indicates a poor overall survival rate, with a hazard ratio value of 0.99 (95% CI: 0.64-1.34, $P < 0.05$).

Conclusions Low expression of microRNA let-7 is closely related to the poor prognosis of NSCLC, so microRNA let-7 may become a new biomarker to evaluate the prognosis of NSCLC patients.

Keywords MicroRNA let-7; Non-small cell lung cancer; Prognosis; Overall survival; Meta-analysis

Background

Lung cancer is one of the most common malignant tumors in the world. It is also the leading cause of mortality in men and women[1]. Non-small cell lung cancer (NSCLC) accounts for the majority of lung cancer. Despite a better understanding of the pathogenesis of NSCLC and the development of effective therapies including surgical treatment, radiotherapy, chemotherapy and targeted therapy, the overall 5-year survival is extremely low (~15%) [2, 3]. Even when treatment is initiated early in the disease, patients showed high recurrence rates, underscoring the need for a deeper understanding of the biology of NSCLC and the identification of more effective biomarkers.

MicroRNAs, approximately 20-25 nucleotides in length, are short and non-coding RNA molecules that negatively regulate gene expression at the post-transcriptional level [4]. MicroRNAs are encoded by specific genes and play an important role in inhibiting mRNA translation or promoting mRNA degradation [5, 6]. MicroRNAs take effects in many biological processes, such as inflammation, cell growth, apoptosis, development, differentiation, endocrine homeostasis, and even cancer [7, 8]. MicroRNA let-7 is a class of microRNAs. Studies have shown that microRNA let-7 family was less expressed in tumor tissues than normal counterparts [9], indicating that microRNA let-7 family members are crucial in the development of cancer.

To elucidate the role of microRNA let-7 as a prognostic biomarker, we performed a meta-analysis. This meta-analysis analyzed relevant studies to provide credible results on whether microRNA let-7 is a reliable prognostic biomarker for NSCLC patients.

Methods

Search strategy

Two researchers performed a literature search from the online databases PubMed, Web of Science, Medline and the Cochrane Library to assess the role of microRNA let-7 in the prognosis of NSCLC. We selected English literature on human subject published before February 16, 2020. The keywords included "let-7," "microRNA," "miRNA," "non-small cell lung cancer" in combination with "NSCLC". We also manually searched the reference lists of the eligible literature to find more potential relevant studies.

Criteria for inclusion and exclusion

All included studies had to meet the following criteria: (1) research subjects were NSCLC patients; (2) studies should investigate the association between microRNA let-7 expression level and overall survival of the patients; (3) relevant data information should be provided. If one or more of the following criteria were met, the literature was excluded: (1) animal experiments or fundamental investigation; (2) the study did not emphasis on NSCLC; (3) no survival outcomes or reported results could not be calculated; (4) duplicate publications or articles in the form of reviews, case reports, editorials, letters, and annals.

Quality assessment and data extraction

To ensure the quality of the meta-analysis, two researchers independently

evaluated the quality of the selected studies using the guideline of the Newcastle-Ottawa Quality Assessment Scale (NOS) [10]. Each study had a score on a scale of 0 to 9, and the study with a score higher than 6 was considered as high quality.

According to the selection criteria and quality assessment, relevant data were extracted from each study, including the name of the first author, year of publication, country of study, number of the research objects, type of microRNA let-7 family, sample source, detecting method, follow-up time, survival analysis data, cutoff point, hazard ratio (HR) values, and 95% confidence intervals (CI). For the literature that only provided Kaplan-Meier curves, we extracted data from the survival graphs and the HR was estimated using the related statistical methods [11]. Two researchers independently extracted the data with cross-check and mutual agreement, and controversial studies were settled by a third researcher.

Statistical analysis

Stata 15.1 (StataSE 15, USA) was used to perform statistical analysis. The effects of HR and 95% CI on microRNA let-7 expression level and NSCLC prognosis were evaluated. For unknown HR and 95% CI, data were calculated from the survival curves using Engauge Digitizer 10.8. Heterogeneity among the studies were assessed by Cochran's Q test (P-values ≤ 0.05 was considered statistically significant) and Higgins's I^2 statistics (the values of 25, 50, and 75% represented low, medium, and high heterogeneity, respectively). When there was statistically significant heterogeneity (P < 0.05 , $I^2 > 50\%$), the random effect model was used, otherwise the fixed effect model

was applied. Publication bias was described by the funnel plots with the Begg and Egger's regression test. $P < 0.05$ was considered statistically significant. Meanwhile, sensitivity analysis was performed to validate the stability of the results.

Results

Characteristics of the included studies

The results of literature retrieval were shown in Fig. 1. A total of 476 studies on microRNA let-7 and NSCLC prognosis were identified by screening PubMed, Web of Science, Medline, Cochrane Library and other sources. Ten duplicates were excluded. A further 425 were removed after screening the titles and abstracts. The remaining 51 were evaluated in full-text reading. And 40 were excluded for the reasons that 9 were non-human studies; 14 lacked critical survival data; and 17 were irrelevant to specific researches. Therefore, 11 studies were included in the meta-analysis [12-22].

The main characteristics of the included studies, which were arranged by ascending order of the year of publication, were listed in Table 1. A total of 2278 NSCLC patients were included in the meta-analysis. Among the 11 studies from included articles, 6 were performed on Asian populations and 5 were on Caucasian populations. All studies detected the microRNA let-7 expression in tissues by quantitative real-time polymerase chain reaction (qRT-PCR) assay. The cutoff points of the expressions were different. All studies analyzed the correlation between microRNA let-7 and overall survival, among which only 9 researches directly showed HR values and 95% CI, while we calculated HR values and 95% CI of the other 2 articles. In

addition, the quality assessment of included studies were shown in Table 1 and the medium NOS score was 7, indicating that all eligible studies were of high quality.

Outcomes from eligible studies

The meta-analysis was conducted to investigate the prognostic role of microRNA let-7 in NSCLC patients. Data analysis using Stata 15.1 statistical software showed statistical heterogeneity between the studies ($P = 0.000$, $I^2 = 91.4\%$). Therefore, we used the random effect model to calculate the parameters included in the studies. The low microRNA let-7 expression was associated with poor overall survival (HR = 0.99, 95% CI: 0.64-1.34, $P < 0.05$, Fig. 2).

Subgroup analysis

A significant heterogeneity was observed in the meta-analysis, and subgroup analyses were conducted to explore the source of heterogeneity. We performed subgroup analyses based on ethnicity and follow-up time. The results of each subgroup analysis were shown in Fig. 3 and 4.

Publication bias and sensitivity analysis

Begg and Egger's regression were conducted to assess the likelihood of publication bias in this meta-analysis. Begg's regression test showed $Pr > |z| = 0.876 > 0.05$, Egger's regression test showed $P > |t| = 0.657 > 0.05$ (Fig. 5 and 6). The results were not statistically significant, suggesting that the probability of publication bias was low.

Sensitivity analysis refers to analyzing the impact of each study on the stability of meta-analysis results by omitting any single study in sequence. Sensitivity analysis showed that the pooled results were stable in this meta-analysis (Fig. 7).

Discussion

MicroRNAs bind to specific target mRNAs by base pairing and negatively regulate gene expression or inhibit translation by degrading target mRNA. This regulation of gene expression plays a critical role in development, cell differentiation and apoptosis [23]. The association between abnormal microRNAs expression and the prognosis of lung cancer, especially NSCLC, has been evaluated in a number of meta-analysis studies. MicroRNA let-7 is a member of the microRNA family and serves as a sequential control factor in determining cell fate [12]. This study summarized the latest researches on microRNA let-7 in NSCLC, focusing on the role of microRNA let-7 as a biomarker in the prognosis of NSCLC patients.

According to Xia et al. [16], the low expression of microRNA let-7 is closely related to the prognosis of NSCLC. More samples of publications have been included in our work. We demonstrated that the low microRNA let-7 expression is statistically associated with overall survival. The HR of low microRNA let-7 expression in NSCLC was 0.99, indicating that low let-7 expression is associated with poor prognosis of NSCLC. As we can see from Fig. 2, the significant heterogeneity was found in the study. We therefore performed subgroup analyses of selected articles based on ethnicity and follow-up time. They proposed different subtotal HR values and found heterogeneity in

subgroups. This may explain the source of heterogeneity in the meta-analysis.

This meta-analysis was designed to investigate the role of microRNA let-7 in the prognosis of NSCLC patients, making it a reliable biomarker and providing clinicians with valuable information for the most effective treatment of NSCLC patients and adjusting treatment strategies. Although a systematic and comprehensive meta-analysis has been conducted, there were still limitations remaining. Firstly, the number of patients included in the study was insufficient to obtain more accurate results, and the basic clinical features of the patients were also different, which could mainly explain the heterogeneity. Secondly, due to the lack of accurate HR values in the original papers, we calculated the HR values in the two studies. Studies that cannot calculate HR values have been removed from the analysis, which may be less persuasive. Finally, there was inevitably a publication bias between studies because positive results are more likely to be published, which may affect the results. However, despite there are heterogeneity, biases, and other limitations that make our study somewhat incomplete, the potential of microRNA let-7 as prognostic biomarker cannot be underestimated. More large-scale and standard studies, as well as a better understanding of microRNA let-7's mechanism of action and target, will enable further understanding of the role of microRNA let-7 in NSCLC prognosis and clinical application.

Conclusions

In summary, this meta-analysis demonstrates the role of microRNA let-7 in predicting prognosis in NSCLC patients. Low expression of microRNA let-7 is closely related to

the poor prognosis of NSCLC, so microRNA let-7 may become a new biomarker to evaluate the prognosis of NSCLC patients.

Abbreviations

NSCLC: non-small cell lung cancer; CIs: Confidence intervals; HRs: Hazard ratios;

Acknowledgements

We would like to thank the researchers and study participants for their contributions.

Author contribution

XTZ and HLK collected and analyzed the data, wrote the paper; HLK performed quality assessment and analyzed the data. XFL and GHC conceived and designed this study. All authors reviewed the paper. All authors read and approved the final manuscript.

Conflict of interest

The authors have no conflicts of interest to disclose.

Funding

The study was supported in part by National Natural Science Foundation of China (81801517 to L.X.F.), Medjaden Academy & Research Foundation for Young Scientists (Grant No. MJR20190009) to L.X.F and the fund of “San-ming” Project of Medicine in Shenzhen (NO.SZSM201812088).

Availability of data and materials

All data are included in this article. The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Figure legends:

Figure 1. Methodological flow chart of the meta-analysis.

Figure 2. The forest plot of studies evaluating HRs of microRNA let-7 in the prognosis of NSCLC.

Figure 3. Meta-analysis of subtotal HRs based on different ethnicity in the prognosis of NSCLC patients.

Figure 4. Meta-analysis of subtotal HRs based on different follow-up time in the prognosis of NSCLC patients.

Figure 5. Begg's regression test for publication bias of this meta-analysis.

Figure 6. Egger's regression test for publication bias of this meta-analysis.

Figure 7. Sensitivity analysis to assess the stability of results.

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

Study ID	Country	Sample size	Let-7	Sample source	Analysis platform	Follow-up time	Survival analysis	Cutoff point	Hazard ratio (HR) value	Quality score	Ref
Takamizawa 2004	Japan	143	Let-7	Tissue	qRT-PCR	>5 years	Univariate Cox analysis	Hierarchical Clustering	Reported	8	12
Yanaihara 2006	USA	52	Let-7a-2	Tissue	qRT-PCR	>1 years	Kaplan-Meier analysis	mean	Reported	7	13
Yu 2008	China	112	Let-7a	Tissue	qRT-PCR	2 years	Kaplan-Meier analysis	median	Reported	8	14
Landi 2010	USA	290	Let-7e	Tissue	qRT-PCR	5 years	Kaplan-Meier analysis	median	Reported	8	15
Xia 2010	China	31	Let-7	Tissue	qRT-PCR	3 years	Kaplan-Meier analysis	median	Calculated	7	16
Voortman 2010	USA	639	Let-7a	Tissue	qRT-PCR	8 years	Multivariate Cox analysis	median	Reported	7	17
Zhang 2012	China	51	Let-7e	Tissue	qRT-PCR	>2 years	Kaplan-Meier analysis	median	Reported	8	18
Jusufovic 2012	Bosnia and Herzegovina	50	Let-7b	Tissue	qRT-PCR	1 years	Kaplan-Meier analysis	median	Reported	7	19
Capodanno 2013	Italy	55	Let-7g	Tissue	qRT-PCR	8 years	Kaplan-Meier analysis	median	Calculated	7	20
Zhao 2014	China	94	Let-7c	Tissue	qRT-PCR	6 years	Kaplan-Meier analysis	median	Reported	7	21
Shin 2016	Korea	761	Let-7a-2	Tissue	qRT-PCR	5 years	Kaplan-Meier analysis	median	Reported	6	22

Figures

PRISMA 2009 Flow Diagram

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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Figure 1

Methodological flow chart of the meta-analysis.

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Begg's regression test for publication bias of this meta-analysis.

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Egger's regression test for publication bias of this meta- analysis.

Figure 7

Sensitivity analysis to assess the stability of results.