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Original Article

The clinical application value of miR-1269 as an unfavorable prognostic indicator of lung cancer

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Abstract: Background: Thanks to microRNAs (miR), a myriad of outstanding achievements have been made in multiple fields in recent years. miR-1269, a newly discovered miR, presents high expression profiles in lung cancer (LC), but its clinical implications in LC have not been clarified yet. Methods: The miR-1269 expressions in the peripheral blood of LC patients, benign pulmonary disease (BPD) patients, and healthy controls were measured using qRT-PCR. Receiver operating characteristic (ROC) curves were employed for the identification of the diagnostic value of miR-1269 in LC, as were Kaplan-Meier (K-M) analyses and a Cox regression model to determine miR-1269's prognostic value in LC. Results: qRT-PCR revealed higher miR-1269 expressions in the LC patients than in the BPD patients and the controls ($P < 0.001$). The LC patients with high miR-1269 expressions had advanced tumor stages (III-IV) and an increased probability of lymph node metastasis (LNM) ($P < 0.01$). Also, evidently elevated miR-1269 levels were observed in the peripheral blood of patients with the advanced tumor stages (III-IV) and LNM. Via ROC curves, we found that miR-1269 is of high clinical significance in the diagnosis of LC and advanced tumor stages. Our K-M survival analysis revealed a lowered 5-year survival rate in patients with high miR-1269 expressions, and our Cox regression analysis found that miR-1269 is an independent prognostic factor for LC. Conclusions: miR-1269, with high expression profiles in LC, indicates unfavorable patient prognoses, so it may be a viable diagnostic and prognostic indicator of LC.

Keywords: miR-1269, lung cancer, prognosis, clinical value, analysis

Introduction

Driven by the improvement in people's living standards and the disorder of people's diets and daily routines, the incidence of cancer keeps increasing yearly [1]. Cancer statistics in 2018 showed that there were 18.1 million new cancer patients and 9.6 million deaths worldwide, among which lung cancer (LC) patients ranked first in terms of both new cases (about 11.6%) and deaths (about 18.4%). Such high morbidity and mortality rates are one of the urgent problems to be solved clinically [2-4]. However, some patients are diagnosed when they are in the disease's advanced stages and have therefore missed the optimal time for an operation when they are admitted to the hospital, because the onset of LC is relatively insidious, the clinical manifestations of the disease are not obvious at the initial stage, and the good clinical diagnostic indicators for this disease are still lacking [5, 6]. Moreover, the long treatment course of advanced LC and the foreseeable unfavorable outcomes aggravates the pressure on the patients and on the family's economic burden to a certain extent [7, 8]. Hence, it is of paramount importance to find a diagnostic index with high specificity and sensitivity to solve this conundrum.

microRNA (miR), a non-coding short-chain RNA about 22nt in length, has become a hot research topic in recent years [9, 10]. It can inhibit target gene translation and transcription by targeting the untranslated region at the 3' end of the downstream target gene mRNA, thus altering target gene expression [11]. Previously, it was shown that miRs are closely associated with tumorigenesis and progression, especially in LC [12, 13]. Multiple published studies have shown that miRs, with differential expressions in LC, are promising diag-

Table 1. Comparison of the clinical data

Variables		Lung cancer group (n=84)	Benign lesion group (n=50)	Control group (n=50)	*P* value
Gender	Male (n=109)	50	28	31	0.828
	Female (n=75)	34	22	19	
Age (years old)	≥ 60 (n=)	55	30	35	0.575
	< 60 (n=)	29	20	15	
BMI (kg/m^2)		22.45±1.84	22.84±2.56	23.22±2.12	0.127

nostic and prognostic markers of LC [14, 15]. Of these, miR-1269, located on the human 4q13.2 chromosome, has been indicated in early studies to show a high expression in LC, and it can inhibit the proliferation and metastasis of LC cells by modulating tp53 and caspase-9 [16]. Also, there is evidence showing that miRs are feasible diagnostic and prognostic markers for tumors [17], but the significance of miR-1269 in the diagnosis and prognosis of LC remains unclear at the present stage.

In view of this, this paper mainly explores the diagnostic and prognostic significance of miR-1269 in LC, hoping to provide potential diagnostic and prognostic indicators for LC.

Methods and materials

Clinical data

Eighty-four LC patients (the lung cancer group) admitted to the Guangrao County People's Hospital between January 2013 and April 2014 were recruited as the research cohort, and concurrently, 50 patients with benign pulmonary disease (BPD) were recruited as the benign lesion group, and 50 healthy check-up patients were recruited the control group. The three groups were comparable in terms of age and sex (P > 0.05, **Table 1**). Tumor tissues were collected intraoperatively, transported in liquid nitrogen, and stored at -80°C. This study was approved by the ethics committee of Guangdong County people's Hospital, and all the subjects or their immediate family members signed the informed consent forms.

Inclusion and exclusion criteria

Inclusion criteria: Some of the collected samples were sent to the Department of Pathology of Guangrao County People's Hospital, where they were diagnosed with non-small cell lung cancer (NSCLC) by two senior pathologists. All the patients had not received any anti-tumor therapy before their diagnoses. This study was approved by the hospital Medical Ethics Committee, and it was conducted in line with the Declaration of Helsinki [18].

Exclusion criteria: Patients with other tumors, patients who were uncooperative with the follow-up, patients with incomplete clinical data, and patients with a life expectancy of less than 3 months.

qRT-PCR detection

The peripheral blood from all the samples was collected and centrifuged at 3000 rpm for 10 min after standing for 30 min for the serum collection. Then the total RNA was extracted from the patient's serum using EasyPure miRNA Kits (TransGen Biotech, Beijing, China), and its purity, concentration, and integrity were determined. cDNA was obtained by reverse transcription using the TransScript Green miRNA Two-Step QRT-PCR SuperMix Kit (TransGen Biotech, Beijing, China), and then amplified using the ABI 7500 PCR instrument (ABI, USA). The reaction system and conditions were prepared and carried out following the kits' instructions. Each sample was provided with 3 duplicate wells, and the experiment was run in triplicate. In this experiment, U6 was used as the internal reference for the data analysis using the $2^{-\Delta\Delta CT}$ [19].

Follow-up

All the patients underwent telephone and outpatient reexaminations and were followed-up for 5 years to record their survival. The patients were followed up every 3 months in the first year and then every 6 months.

Database validation

We logged in to starBase (http://starbase.sysu.edu.cn/) [20] to analyze the miR-1269 expres-

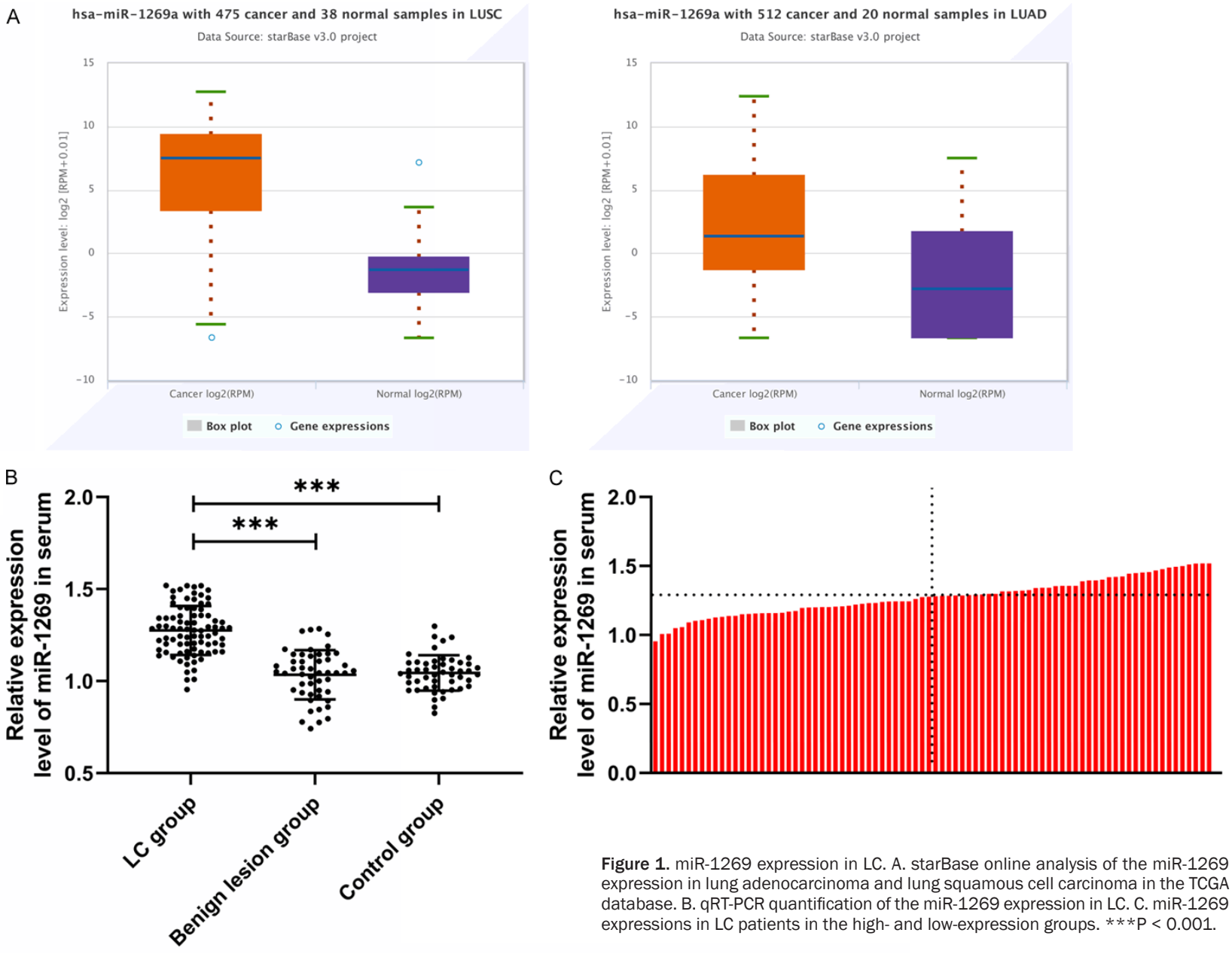

Figure 1. miR-1269 expression in LC. A. starBase online analysis of the miR-1269 expression in lung adenocarcinoma and lung squamous cell carcinoma in the TCGA database. B. qRT-PCR quantification of the miR-1269 expression in LC. C. miR-1269 expressions in LC patients in the high- and low-expression groups. ***P < 0.001.

Table 2. The relationship between miR-1269 and the clinical data of the LC patients

Variables		Relative expression of miR-1269		*P* value
		Low expression group (n=42)	High expression group (n=42)	
Gender	Male (n=50)	22	28	0.182
	Female (n=34)	20	14	
Age (years old)	≥ 60 (n=55)	29	26	0.491
	< 60 (n=29)	13	16	
Tumor size (cm)	≥ 3 (n=48)	22	26	0.378
	< 3 (n=36)	20	16	
Pathological type	Lung squamous cell carcinoma (n=42)	20	22	0.663
	Lung adenocarcinoma (n=42)	22	20	
Neoplasm staging	I-II (n=41)	27	14	0.005
	III-IV (n=43)	15	28	
Lymph node metastases	Yes (n=31)	9	22	0.003
	No (n=53)	33	20	

sions in lung adenocarcinoma (LAC) and lung squamous cell carcinoma (LSCC) in the TCGA data, and we downloaded the histogram file for display.

Statistical analyses

In this study, the SPSS 20.0 software package was utilized for the Cox regression analysis, and the GraphPad 7 software package was used for the T, Chi-square, and Kaplan-Meier (K-M) tests. The inter-group comparisons were performed using independent sample t tests, (represented by T), the multi-group comparisons using one-way ANOVA (denoted by F), and the post-hoc pairwise comparisons using LSD-t tests. The diagnostic value of miR-1269 in LC was drawn using receiver operating characteristic (ROC) curves, the 3-year survival of patients was plotted using K-M survival curves and analyzed using log-rank tests. Multivariate Cox regression analyses were employed to analyze the independent risk factors influencing the patient prognoses. The level of significance was taken as $P < 0.05$.

Results

miR-1269 is increased in the serum of LC patients

To determine the miR-1269 expression in LC, we first used the starBase online website to analyze its expression profiles in LAC and LSCC in the TCGA database. The results indicated that miR-1269 is elevated in both LAC and LSCC, and it was higher than it was in the controls ($P < 0.001$, **Figure 1A**). Further, we utilized qRT-PCR to verify the expressions of miR-1269 in LC. miR-1269 was shown to be observably elevated in the lung cancer group as compared to the control group and the benign lesion group ($P < 0.001$), but there were no significant differences between the latter two groups ($P > 0.05$, **Figure 1B**). Furthermore, the LC patients were further subdivided high- and low-expression groups on the basis of the median miR-1269 value (**Figure 1C**), so as to analyze the correlations of miR-1269 with the patient clinical data. The results identified a notably increased incidence of advanced tumor stages (III-IV) and LNM in the patients in the high expression group ($P < 0.01$, **Table 2**).

Diagnostic value of miR-1269 in LC

We have determined the expression of miR-1269 in LC through the above studies. To further analyze its diagnostic value in LC, we included additional indicators of clinical data that showed differences with miR-1269. Our qRT-PCR analysis revealed that miR-1269 presents with lower levels in patients in stages I-II than in patients in stages III-IV ($P < 0.01$, **Figure 2A**), and higher levels in patients with lymph node metastases than in those without ($P < 0.01$, **Figure 2B**). Then, we drew the ROC curves based on the factors with differences, and through our analysis, we found that miR-1269 has a diagnostic value in LC (**Figure 2C**),

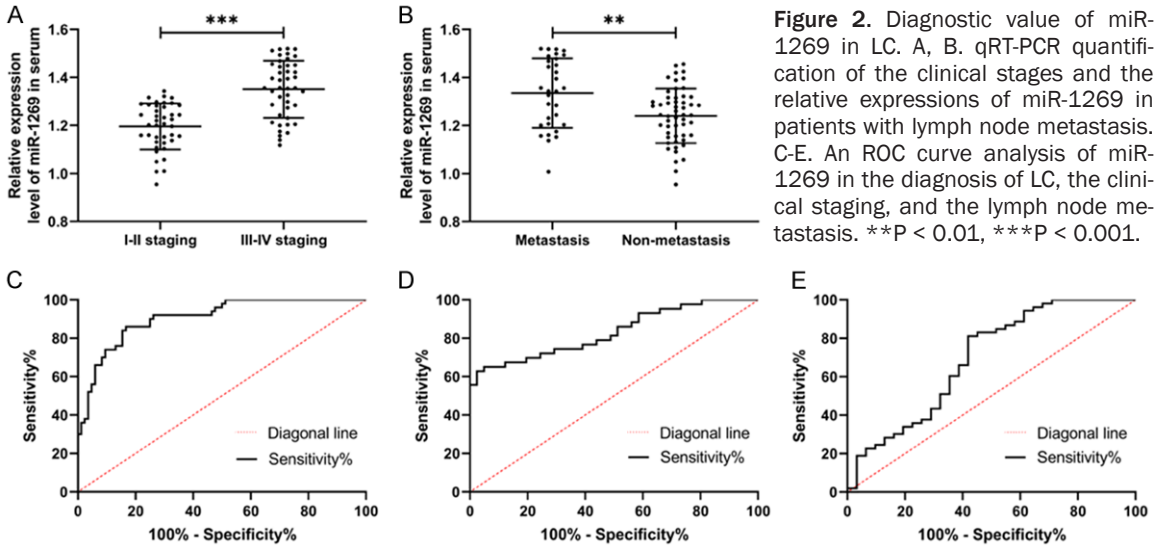

Figure 2. Diagnostic value of miR-1269 in LC. A, B. qRT-PCR quantification of the clinical stages and the relative expressions of miR-1269 in patients with lymph node metastasis. C-E. An ROC curve analysis of miR-1269 in the diagnosis of LC, the clinical staging, and the lymph node metastasis. **P < 0.01, ***P < 0.001.

Table 3. ROC parameters

Variables	AUC	95% CI	*P* value	Specificity	Sensitivity	Youden index	Cut off
LC	0.906	0.856-0.956	< 0.001	83.33%	86.00%	69.33%	< 1.152
Neoplasm staging	0.832	0.745-0.918	< 0.001	97.56%	62.79%	60.35%	> 1.324
Lymph node metastases	0.694	0.570-0.818	0.003	58.06%	81.13%	39.19%	< 1.324

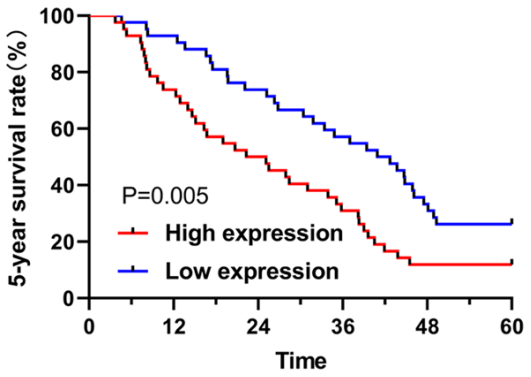

Figure 3. An analysis of the miR-1269 expressions and a 5-year survival analysis of LC patients.

in neoplasm staging (**Figure 2D**), and in LNM (**Figure 2E**; **Table 3**).

Patients with high miR-1269 expression have unfavorable prognoses

At the end of the study, we analyzed the correlation of miR-1269 with the prognoses of the LC patients. After 3 years of follow-up, we found that the 5-year survival rate of the patients with high miR-1269 expressions had decreased significantly (**Figure 3**). A Cox regression analysis showed that miR-1269, neoplasm staging, and LNM were the prognostic factors for LC patients, while a multivariate analysis found that miR-1269 and LNM were independent prognostic factors for LC patients (**Table 4**).

Discussion

LC is currently the malignancy with the highest morbidity and mortality across the globe. Therefore, effective diagnostic schemes and viable prognostic indicators are vital in the treatment process [21]. In this paper, we confirmed increased miR-1269 levels in LC through our experiments, and patients with high miR-1269 expressions had unfavorable outcomes. Apart from that, miR-1269 can be a potential diagnostic index for LC, neoplasm staging, and LNM.

miR, a hot research topic in various disciplines, has been constantly improved by the clarification of its mechanism and functions [22]. Early studies showed that abnormally expressed miRs can activate the expression of oncogenes and inhibit the expression of tumor suppressor genes to create an imbalance in the cells of the affected organism, which ultimately leads to

Table 4. Cox regression analysis

Variables	Univariate			Multivariate		
	P value	HR value	95% CI	*P* value	HR value	95% CI
Gender (male *VS* female)	0.255	0.754	0.463-1.226			
Age (≥ 60 *VS* < 60)	0.450	0.822	0.494-1.367			
Tumor size (≥ 3 *VS* < 3)	0.861	0.958	0.592-1.551			
Pathological type (lung adenocarcinoma *VS* lung squamous cell carcinoma)	0.376	0.806	0.499-1.300			
Neoplasm staging (I-II *VS* III-IV)	0.009	1.918	1.176-3.126	0.137	1.485	0.882-2.500
Lymph node metastases (metastasis *VS* no metastasis)	0.003	0.476	0.291-0.778	0.009	0.514	0.312-0.846
miR-1269 (high expression *VS* low expression)	0.006	1.973	1.216-3.202	0.015	1.835	1.123-3.000

cancer [22-24], suggesting that miRs can be feasible diagnostic markers for tumors. Compared with tumor tissues, peripheral blood collection is non-invasive, which reduces the harm to patients and facilitates the collection. Guo et al. [25] showed that the serum expressions of miR-1915-3p and miR-455-3p in breast cancer patients can function as biomarkers to determine breast cancer.

As a recently discovered miR, miR-1269 is mainly expressed in liver cancer [26] and gastric cancer [27]. In this study, we first found through a database analysis that miR-1269 is increased in LAC and LSCC. Furthermore, qRT-PCR verified that serum miR-1269 was higher in the LC patients than in the BPD patients and controls, showing an upward trend, which is consistent with the results of the database searching and with Bao et al. [16]. Moreover, the patients were subdivided into high- and low expression groups according to their median miR-1269 expressions to observe the correlation of miR-1269 with the patient clinical data. We found that the patients with high miR-1269 expressions had advanced tumor stages and an increased probability of LNM, which agrees with the results of Bao et al. and which was mutually verified. However, their research did not deeply analyze the clinical diagnostic and prognostic roles of miR-1269. But here, we further analyzed its diagnostic and prognostic significance in LC. Through ROC curves, we found that the area under the curve of miR-1269 in diagnosing LC and healthy controls was > 0.9, implying that miR-1269 is a viable diagnostic index for LC. What's more, factors (neoplasm staging and LNM) with differences in their clinical baseline data were selected for further comparisons. It was found that miR-1269 is highly expressed in patients with advanced tumor stages and LNM, and our ROC analysis also demonstrated that miR-1269 possesses a certain value in diagnosing advanced tumor stages and LNM. However, miR-1269 is not ideal for distinguishing LNM and LC.

At the end of the study, we analyzed the prognostic value of miR-1269 in LC. After the follow-up, we found that the 5-year survival rate of patients with high miR-1269 expressions was significantly lower than the 5-year survival rate of patients with low expressions. Through a Cox regression analysis, miR-1269 was identified to be an independent prognostic factor for LC. Through these experiments, we can confirm that miR-1269 enjoys a high clinical value in LC.

However, there are still some limitations to this paper. First, further clinical trials are warranted to determine whether miR-quantification can be widely used in clinical practice. Second, the mechanism of miR-1269 in LC needs to be further clarified. Third, long-chain non-coding RNA (lncRNA) has been shown to be able to sponge miR [28], so whether miR-1269 can be regulated by lncRNA needs to be confirmed by further studies. Finally, the number and types of samples collected in this study were small and narrow. Recently, accumulating evidence has found that various samples, such as alveolar lavage fluid [29] and exosomes [30], have a high clinical value in the diagnosis of LC. Therefore, we hope to collect more clinical samples, carry out further clinical experiments and conduct bioinformatics analyses in our future research, so as to improve our research conclusions.

Taken together, miR-1269, with its high expression profiles in LC, indicates an unfavorable prognosis in patients, so it is expected to be a viable diagnostic and prognostic indicator of LC.

Disclosure of conflict of interest

None.

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