miR-181 Expression is Associated With The Prognosis in Lung Cancer.

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Abstract

Background: Lung cancer is one of the most common cancers, with high morbidity and mortality. MiRNAs are proved to play important roles in various human cancers. In our study, we aimed to explore the prognostic value of *miR-181* in lung cancer

Methods: Quantitative real-time polymerase chain reaction (QRT-PCR) was used to detect the expression level of *miR-181* in lung cancer tissues and the paired non-cancerous tissues. The relationship between *miR-181* expression and clinicopathologic parameters were analyzed by chi-square test. Kaplan-Meier method with log rank test was applied for overall survival analysis. Furthermore, the Cox regression analyses were performed to evaluate the prognostic value of *miR-181* in lung cancer.

Results: Down-regulated *miR-181* expression was observed in lung cancer tissues (P<0.001), moreover, its expression was significantly correlated with TNM stage (P=0.015) and metastasis (P=0.000). In addition, lung cancer patients with lower *miR-181* expression level had poorer overall survival than those with higher expression (log rank test, P=0.011). Cox regression analysis suggested that *miR-181* was an independent prognostic factor for lung cancer (HR=1.961, 95%CI=1.135-3.388, P=0.016).

Conclusion: *MiR-181* may be a tumor suppressor gene in lung cancer, which can predict outcomes for the patients.

Background

Lung cancer is one of the most common cancers and the leading causes for cancer-related death around the world [1]. In our country, the mortality of lung cancer shows a trend of rapid growth in the past three decades, becoming a major public health problem [2]. Lack of effective biomarkers for early detection and prognosis may be responsible for the poor survival rate [3]. The pathogenesis of lung cancer is a complex progression and remains unclear. It is general accepted that the accumulation of genetic and epigenetic alterations is the baseline mechanism for lung cancer development, with the involvement of numerous of oncogenes and tumor suppressor genes. Therefore, we deduced that the molecules might serve as noninvasive biomarkers for lung cancer prognosis, which could accurately predict tumor progression for the patients.

MicroRNAs (miRNAs) are a class of small, noncoding RNAs with about 22 bp length which regulate gene expression at post transcriptional level [4]. MiRNAs have been confirmed to play important roles in various biological processes, including cell proliferation, differentiation, apoptosis, and diseases initiation [5]. Recent studies showed that miRNAs were significantly correlated with tumor progression, via serving as oncogenes and suppressor genes in carcinogenesis [6]. Growing number of miRNAs were proved to take part in the initiation, development, chemoresistance, and recurrence of lung cancer, such as *miR-1207-5p*, *miR-184*, *miR-137* and so on [7–9]. In the present study, we evaluated the clinical significance of *miR-181* in lung cancer prognosis.

MicroRNA-181 (*miR-181*) was initially identified in neuronal cells, and then observed in other systems [10]. The *miR-181* family contains four highly conserved members: *miR-181a*, *miR-181b*, *miR-181c* and *miR-181d.* Among the four family members, *miR-181a-1* and *miR-181b-1* locate on chromosome 1, *miR-181a-2* and *miR-181b-2* are on chromosome 9, and *miR-181c* and *miR-181d* locate on chromosome 19 [11]. Recently, the dysregulation of *miR-181* was observed in several cancers, including prostate cancer, acute myeloid leukemia, and glioma [12–14]. The expression levels of *miR-181* in lung cancer were also detected in the previous studies. In the study of Huang et al., the down-regulated *miR-181* was observed iin non-small cell lung cancer (NSCLC), suggesting its antitumor effects [15]. However, the prognostic value of *miR-181* in lung cancer was rarely investigated.

In this study, we aimed to explore the relationship between clinical characteristics and *miR-181* expression, as well as its prognostic value in lung cancer patients.

Materials And Methods

Patients and specimens collection

The lung cancer tissue samples and paired non-cancerous tissue specimens were collected from 131 lung cancer patients who were diagnosed by pathologists in Cangzhou Central Hospital. None of the lung cancer patients received chemotherapy or radiotherapy before sampling. All the tissue samples were immediately frozen in liquid nitrogen and then stored at -80℃ until RNA extraction. Our study was approved by the ethics committee of Cangzhou Central Hospital, and the written consents were obtained from all patients and their families. The lung cancer patients enrolled in a five-year investigation. The clinicopathological features were listed in Table 1, including age, sex, tumor size, diseased region, pathologic types, TNM stage, and metastasis.

Table 1
Association of *miR-181* expression with clinicopathological features of lung cancer patients

Feathers	No.	*MiR-181* expression		*P* values
	N = 131	Low (n = 79)	High (n = 52)	
Age (years)				
< 60	60	39	21	0.313
≥ 60	71	40	31	
Gender				
Male	80	46	34	0.411
Female	51	33	18	
Tumor size				
< 3 cm	65	40	25	0.775
≥ 3 cm	66	39	27	
Diseased region				
central type	57	35	22	0.822
peripheral	74	44	30	
Pathologic types				
SC	32	19	13	0.365
AC	39	27	12	
NSCLC	60	33	27	
TNM stage				
I-II	66	33	33	0.015
III-IV	65	46	19	
Metastasis				
yes	52	41	11	0.000
no	79	38	41	
Note: SC: squamous carcinoma; AC: adenocarcinoma; NSCLC: non-small cell lung cancer				

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

The total RNA of the tissue samples was isolated with Trizol (Invitrogen) following manufacturer's instructions. The first-strand cDNA was synthesized by the Taqman™ miRNA reverse transcription kit. In our study, the relative expression of *miR-181* was evaluated by qRT-PCR which was performed with SYBR Green Premix Ex Taq (Takara, Dalian, China). *U6* small RNA served as internal control. The primer sequences of *miR-181* and *U6* were: *miR-181* forward: 5'-AGTGAACATTCAACGCTGTCGGT-3', reverse: 5'-GCCATAGGGTACAATCAACGGTCG-3'; *U6* forward: 5'-CTCGCTTCGGCAGCACA-3', reverse: 5'-AACGCTTCACGAATTTGCGT-3'. The expression levels of *miR-181* were calculated by $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

All statistical analyses were performed using the software of SPSS (SPSS 19.0) and GraphPad Prism 5. The expression level of *miR-181* was expressed as mean ± SD and analyzed by student's t test. Chi-square test was applied to analyze the associations between *miR-181* expression and clinicopathological characteristics in lung cancer patients. The Kaplan-Meier method with log rank test was performed to estimate overall survival. Cox regression analysis was used to investigate the prognostic significance of *miR-181* in lung cancer. $P < 0.05$ was considered statistically significant in this study.

Results

Baseline characteristics of the study subjects and their expression elvels of *miR-181*

131 lung cancer patients including 80 men and 51 women were collected in the present study, with the average age of 55.6 years. The clinical characteristics of the study subjects were listed in **Table 1**. QRT-PCR was performed to examine the *miR-181* expression in lung cancer tissues and paired non-cancerous tissues. In **Figure 1**, the results showed that *miR-181* expression was significantly lower in lung cancer tissues than that in matched non-cancerous tissues ($P<0.001$).

The relationship between *miR-181* expression and clinicopathologic features in lung cancer

In order to evaluate the correlation between *miR-181* levels and clinicopathologic characteristics, the lung cancer patients were dived into high expression group (n=52) and low expression group (n=79) according to their average expression level of *miR-181*. The results in **Table 1** revealed that the expression of *miR-181* was obviously related with TNM stage ($P=0.015$) and metastasis ($P=0.000$). However, there was no significant association between *miR-181* levels and age, gender, diseased region, tumor size or pathologic types in lung cancer patients (all $P>0.05$).

Overall survival analysis

Kaplan-Meier with log rank test was applied to estimate overall survival of the patients according to their expression levels of *miR-181*. Analysis results showed that lung cancer patients with low *miR-181* expression had worse overall survival rates than those with high expression (log-rank test $P=0.011$) (**Figure 2**).

Prognostic value of *miR-181* expression in lung cancer

In order to estimate the prognostic value of *miR-181* in lung cancer patients, we used Cox regression analysis. Univariable analysis suggested that *miR-181* (HR=1.961, 95%CI=1.135-3.388, *P*=0.016) and TNM stage (HR=1.671, 95%CI=1.033-2.704, *P*=0.036) were significantly correlated with lung cancer prognosis. In addition, multivariate analysis indicated that *miR-181* was an independent prognostic factor for patients with lung cancer (HR=1.961, 95%CI=1.135-3.388, *P*=0.016) (**Table 2**).

Table 2
Cox analyses for *miR-181* in 131 lung cancer patients

Characteristics	Univariable analysis		Multivariate analysis	
	HR(95%CI)	*P*	HR(95%CI)	*P*
MiR-181 (low vs high)	1.961 (1.135–3.388)	0.016	1.961 (1.135–3.388)	0.016
Age (≥ 60 vs < 60)	1.122 (0.693–1.816)	0.641	-	-
Gender (male vs female)	1.323 (0.811–2.159)	0.262	-	-
Tumor size (≥ 3 cm vs < 3 cm)	1.244 (0.774-2.000)	0.367	-	-
Diseased region (central type vs peripheral)	0.743 (0.458–1.207)	0.230	-	-
Pathologic types (SC vs NSCLC)	0.966 (0.532–1.753)	0.909	-	-
(AC vs NSCLC)	0.826 (0.477–1.431)	0.496	-	-
TNM stage (III-IV vs I-II)	1.671 (1.033–2.704)	0.036	-	-
Metastasis (yes vs no)	1.328 (0.830–2.126)	0.236	-	-
Note: -: indicated no related data.				

Discussion

Lung cancer is a leading cause for death among cancer patients in the world [16]. A variety of therapeutic regimens are applied to lung cancer, including surgery, chemotherapy, radiotherapy, and targeting therapy, but the five-year survival rate of the patients are still unsatisfactory, especially for those at advanced

stage [17, 18]. Therefore, it is necessary to identify a valuable factor to predict tumor progression and guide treatment for lung cancer patients.

Growing evidences demonstrated that miRNAs were critical regulators in tumor progression [19]. MiRNAs could regulate the biological behaviors of cells via interacting with the key genes in the cells, thus involved in the progression and metastasis of cancers [20]. Recently, the effects of *miR-181* in cancers had aroused attentions. The down-regulation of *miR-181* was observed in some cancers, including glioma, chronic lymphocytic leukemia, and oral squamous cell carcinoma [21–23]. While in some other cancers, the expression of *miR-181* was elevated. For example, a study conducted by Tong et al. suggested that up-regulated *miR-181* was observed in prostate cancer, which could promote tumor growth and aggressive progression [12]. Zhan et al. reported that serum levels of *miR-181* were not significantly different between hepatocellular carcinoma patients and healthy individuals, suggesting that it did not involve in the cancer progression [24]. All of the related studies indicated that *miR-181* played various roles in different types of cancer.

In our study, we found *miR-181* expression level was lower in lung cancer tissues than that in the matched noncancerous tissues. In addition, the decreased levels of *miR-181* were significantly correlated with advanced TNM stage, and positive metastasis. The results might reveal that *miR-181* as a tumor suppressor gene could inhibit tumor progression in lung cancer. The conclusion was consisted with the previous studies. Huang et al. reported that *miR-181* was down-regulated in NSCLC tissues and cell lines, moreover, cell experiments suggested that over-expression of *miR-181* could inhibit tumor cell proliferation, migration and invasion, and promote apoptosis [15]. Therefore, we speculated that abnormal expression of *miR-181* might cause alterations in some genes which were related to behaviors of lung cancer cells, leading to tumor progression. The exact mechanisms for *miR-181* regulating lung cancer needed to be investigated in further study.

Based on its functions in tumor progression, *miR-181* family was proved to serve as biomarkers for several cancers. A meta-analysis conducted by Lin et al. showed that *miR-181a/b* were significantly associated with overall survival in patients with hematological malignancies and could act as prognostic markers for these patients [25]. Pichler et al. reported that *miR-181a* expression levels could predictor outcomes in patients with colorectal cancer treated with EGRF inhibitor [26]. In this study, we evaluated the prognostic value of *miR-181* in lung cancer. Survival analysis showed that lung cancer patients with low *miR-181* expression had a poor survival rate. In addition, cox regression analysis further demonstrated that the *miR-181* expression level was an independent prognostic factor for lung cancer patients. Low *miR-181* expression predicted poor prognosis in lung cancer patients. The potential biomarker might improve the management of lung cancer.

Conclusions

In conclusion, down-regulation of *miR-181* predicts aggressive clinical characteristics and poor survival in lung cancer patients. *MiR-181* may be a potentially prognostic biomarker for lung cancer.

List Of Abbreviations

Quantitative real-time polymerase chain reaction (QRT-PCR)

MicroRNAs (miRNAs)

MicroRNA-181 (*miR-181*)

non-small cell lung cancer (NSCLC)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Cangzhou Central Hospital and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.**Competing interests**The authors declare that they have no competing interests.

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Authors' contributions

Y.Z. conceived and designed the experiments; Y.Z. conceived and performed the experiments; X.K. prepared figures. H.W. wrote the main manuscript text. All authors reviewed the manuscript.

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Figures

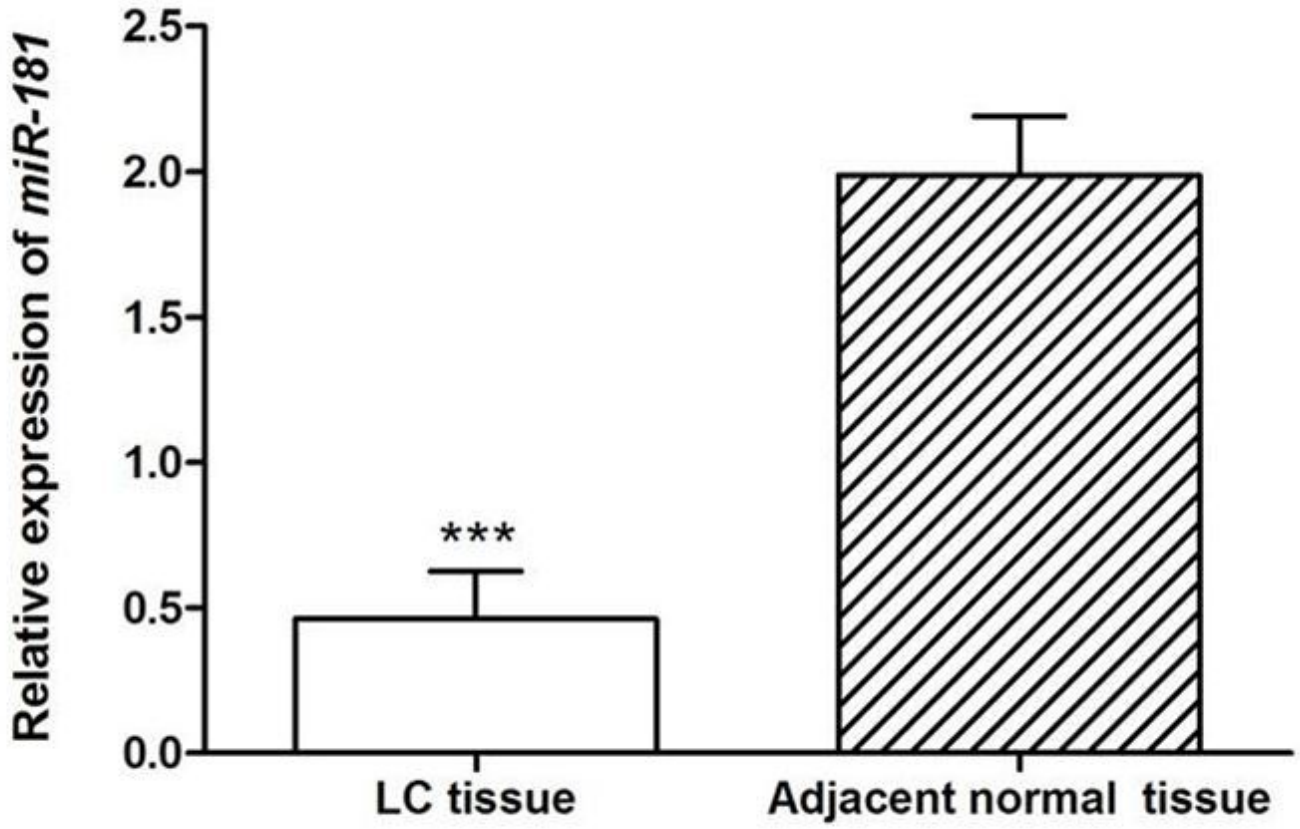

Figure 1

MiR-181 expression level between lung cancer tissues and matched adjacent noncancerous tissues. QRT-PCR results suggested that compared with adjacent normal tissues, the expression levels of miR-181 were significantly down-regulated in lung cancer tissues. ***: indicated P<0.001.

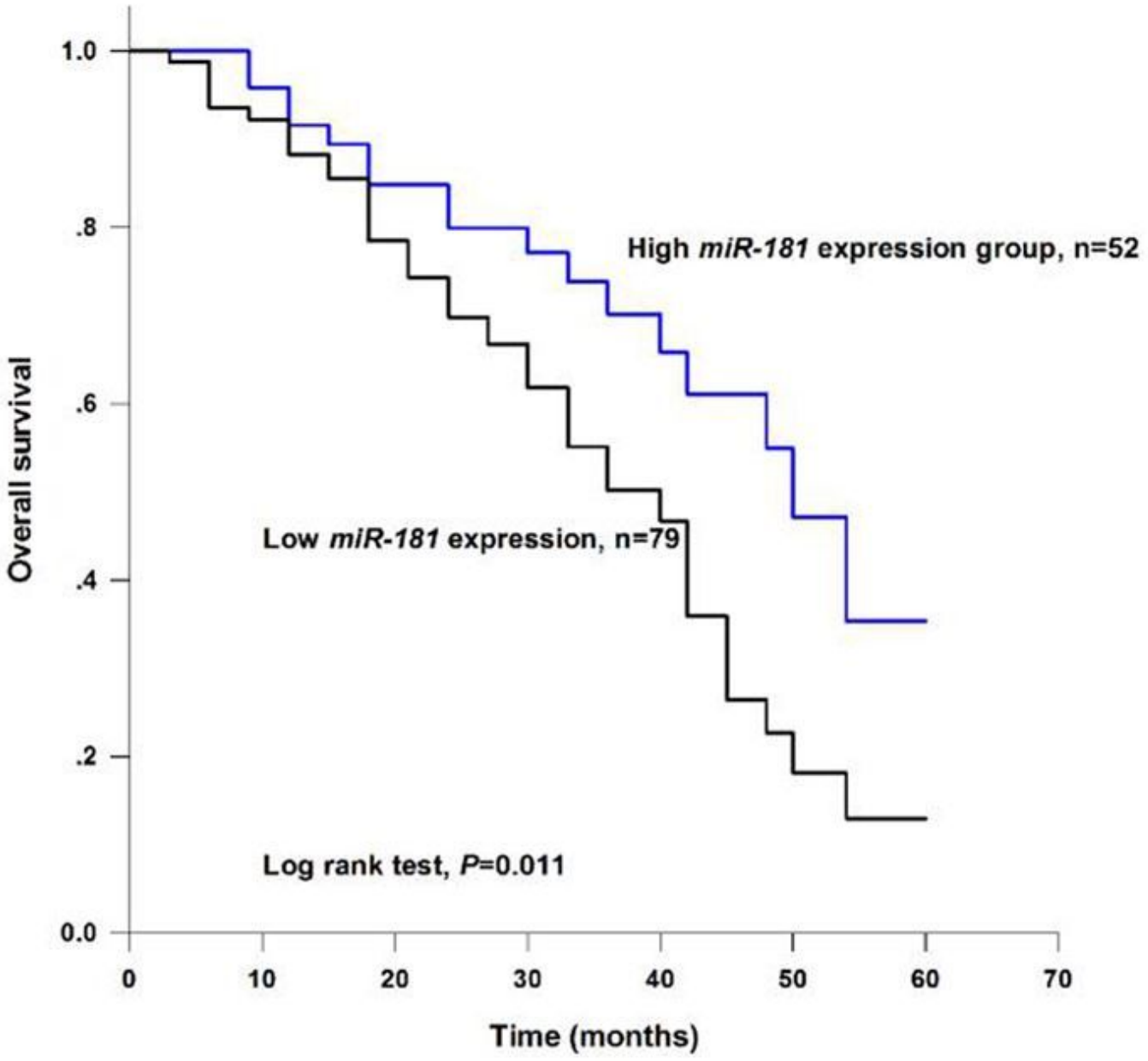

Figure 2

Overall survival analysis for lung cancer patients based on their expression of miR-181. Patients with high levels of miR-181 had a better overall survival than those with low levels (log rank test, P=0.011).