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Expression and Significance of Circulating MicroRNA-31 in Lung Cancer Patients

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
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Background: The aim of this study was to investigate the expression level of circulating microRNA-31(miRNA-31) in lung cancer patients and its clinical significance.

Material/Methods: Real-time fluorescent quantitative PCR was utilized to detect the circulating miRNA-31 expression levels in 300 lung cancer patients and 300 health control subjects. The ROC curve was drawn to evaluate the diagnostic value of the circulating miRNA-31 expression levels in lung cancer. The 300 lung cancer patients were divided into a miRNA-31 low-expression group and a miRNA-31 high-expression group. A survival curve was drawn according to the Kaplan-Meier method to evaluate the prognostic value of the circulating microRNA-31 expression levels for lung cancer.

Results: The circulating miRNA-31 expression levels in the lung cancer patients (l.88±0. 67) increased significantly (P<0.001) compared to the healthy controls (0.58±0. 44). The area under the ROC curve drawn according to the circulating miRNA-31 expression levels was 0.785 (95% CI=0.486–0.763). When the critical value was 1.27, the sensitivity and specificity for lung cancer diagnosis according to the circulating miRNA-31 expression levels were 0.769 and 0.745, respectively. The difference in the survival curve between the miRNA-31 low-expression group (123 cases) and high-expression group (177 cases) was statistically significant (P=0.004). Median survival period of the low-expression group (38.44 months) was longer than that of the high-expression group (25.23 months).

Conclusions: miRNA-31 may be a molecular marker for the diagnostic and prognostic evaluation of primary lung cancer.

MeSH Keywords: Blood Circulation • Lung Neoplasms • RNA Isoforms

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Background

Lung cancer is the malignant tumor with the highest morbidity and mortality around the world. Its incidence is gradually increasing, and the 5-year survival rate is only 15% [1]. MicroRNAs (miRs) are endogenous non-coding RNAs of ~23-mer, which have important roles in regulation of gene expression. The mature form of miRs silence gene expression by binding to the 3'-UTR of target mRNAs and initiate translational repression or cleavage of cognate mRNAs [2]. miRNA-31 was reported to be associated with human cancers [3]. Overexpression of miRNA -31 inhibits cancer cell proliferation by p53-dependent mechanisms in ovarian cancer cells [4]. miRNA -31 was also reported to inhibit metastasis, and it enhanced primary tumor growth in breast cancer [5]. Furthermore, there is a report suggesting that miRNA -31 inhibits cell proliferation, migration, and invasion in malignant mesothelioma [6]. Contributions of miRNA -31 to the activation of hypoxia-inducible factor for development of head and neck squamous cell cancer were also described [7]. Previous studies [8,9] have found high expression of miRNA-31 in lung cancer tissues, but few studies have focused on its expression level in peripheral blood. We collected peripheral blood from primary lung cancer patients, and detected and analyzed the miRNA-31 expression through real-time fluorescent quantitation PCR method to investigate the potential value of miRNA-31 expression level in peripheral blood for early diagnosis and prognosis of lung cancer.

Material and Methods

Subjects

We enrolled 300 primary lung cancer patients treated with carcinectomy in the Department of Respiratory Medicine, Nanjing Medical University Affiliated Nanjing Hospital from May 2005 to December 2014. All primary lung cancer patients without any chemoradiation before surgery were found for the first time and finally diagnosed by pathology. There were 123 cases ≤60 years old and 177 cases were >60 years old; 192 cases were smokers and 108 cases were not smokers; 114 cases were in primary lesion T1 grade, 105 cases were in T2 grade, and 81 cases were in T3 grade; 54 cases were in clinical stage I, 48 cases were in stage II, 75 cases were in stage III, and 123 cases were in stage IV; 72 cases were well differentiated, 111 cases were moderately differentiated, and 117 cases were poorly differentiated; 126 cases had lymph nodes metastasis and 174 cases were without lymph nodes metastasis. All the patients were treated with standard therapy such as chemotherapy and radiotherapy after the operation. A total of 300 health adults were enrolled as controls.

Detection of miRNA-31 in peripheral blood

We collected 5 ml of venous blood from each patient, using EDTA- anticoagulation tubes, before the operation, and centrifuged them at 3000 r/m at 4°C for 10 min, then placed the supernatant into DEPC-treated EP tubes, and stored them at –80°C. We treated the total RNA with a reverse transcription kit (Bioteck, Beijing, China) according to the protocol to obtain cDNA and then performed real-time fluorescent quantitation PCR with an ABI 7500 fluorescent quantitation PCR analyzer (ABI, CA, USA). Taking miRNA-16 as a reference, we calculated the mean of 3 multiple wells for each sample. We used 3 multiple wells for each sample to obtain their mean and calculated the miRNA-31 expression levels with $2^{-\Delta CT}$ ($\Delta CT=CT_{miRNA-31}-CT_{miRNA-16}$).

Statistical analyses

Data were processed using SPSS 21.0 (Chicago, IL, USA). Two independent-samples t-tests were utilized to compare miRNA-31 expression levels in peripheral blood of lung cancer patients and healthy adults. The χ^2 test was used to compare the high-expression rate of miRNA-31 in peripheral blood of patients with different clinical pathology characteristics. We established an ROC curve to evaluate the diagnostic value of miRNA-31 expression level in peripheral blood for primary lung cancer. Taking death as the terminal event after follow-up for 5 years, we drew the survival curve for the miRNA-31 high-expression group and low-expression group using the Kaplan-Meier method and used the log-rank test for the 2 curves with the test size of $P<0.05$.

Results

Comparison of circulating miRNA-31 expression level in peripheral blood of lung cancer patients and health controls

The miRNA-31 expression level in peripheral blood of lung cancer patients (l.88±0. 67) increased significantly ($P<0.001$) compared to the health controls (0.58±0. 44).

Comparison of circulating miRNA-31 expression level in patients with different clinical pathology characteristics

Taking 1.88 – the mean of miRNA-31 expression levels in peripheral blood of 300 lung cancer patients – as critical value, we categorized cases whose peripheral blood miRNA-31 were <1.88 into the low-expression group and that ≥1.88 into the high-expression group; therefore, 177 cases were in the high-expression group and 123 cases were in the low-expression group. The comparison of miRNA-31 expression level in

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Table 1. Comparison of miRNA-31 expression level in peripheral blood of patients with different clinical pathology characteristics.

Group	n	Low-expression	High-expression	P value
Age (years)				0.157
≤60	123	48	75	
>60	177	84	93	
Smoking				**0.001**
Yes	192	69	123	
No	108	63	45	
Grade				0.633
T1	114	45	69	
T2	105	48	57	
T3	81	39	42	
Clinical stages				**<0.001**
I	54	36	18	
II	48	33	15	
III	75	24	51	
IV	123	39	84	
Differentiation				0.444
Well	72	27	45	
Moderately	111	51	60	
Poorly	117	54	63	
Lymph node metastasis				**<0.001**
Yes	126	30	96	
No	174	102	72	

peripheral blood of patients with different clinical pathology characteristics is shown in Table 1.

ROC curve for diagnostic value of miRNA-31 expression level in peripheral blood for primary lung cancer

As shown in Figure 1, the area under the ROC curve was 0.785 (95% CI=0.486–0.763). Sensitivity and specificity for primary lung cancer diagnosis according to the miRNA-31 expression levels in peripheral blood were 0.769 and 0.745, respectively, when the critical value was 1.27.

Relationship between miRNA-31 expression level in peripheral blood of lung cancer patients and their prognosis

The 2 survival curves of miRNA-31 high-expression group and low-expression group are shown in Figure 2; their differences were statistical significance (P=0.004). The median survival period of the low-expression group (38.44 months) was longer than that of the high-expression group (25.23 months).

Discussion

In the present study, we found the circulating miRNA-31 expression levels in the lung cancer patients increased significantly compared to the health controls. Also, median survival period of the low-expression group was longer than that of the high-expression group. Our results indicated that miRNA-31 is a molecular marker for the diagnostic and prognostic evaluation of primary lung cancer.

Early detection and early treatment are effective ways to improve the recovery rate and the prognosis of lung cancer patients [10,11]. Therefore, it is very important to search for markers for early diagnosis and prognosis evaluation. The correlations between miRNA expression and tumor generation,

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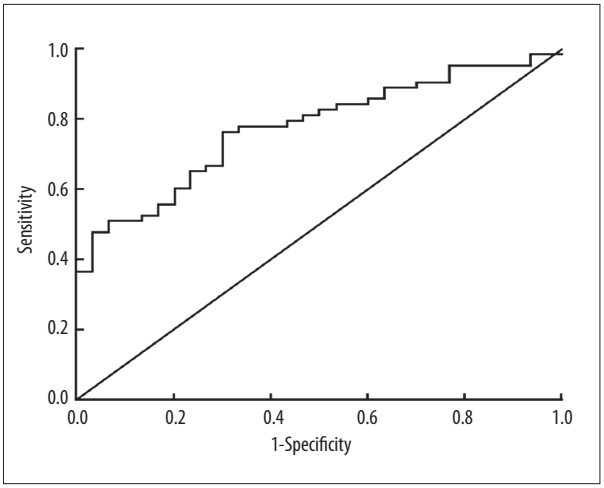

Figure 1. ROC curve for expression of circulating miRNA-31 levels in lung cancer, with an area under the ROC curve=0.785.

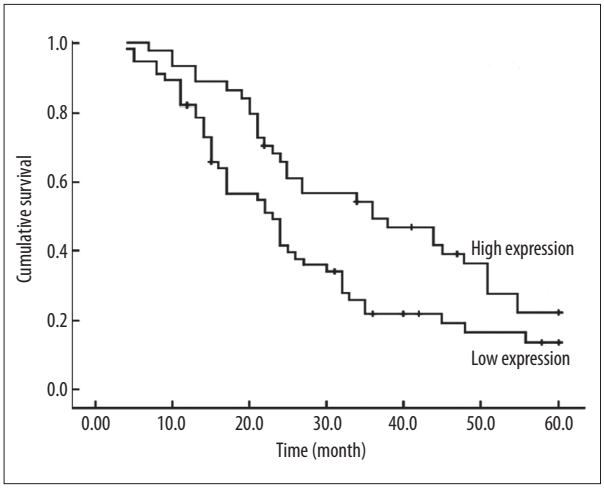

Figure 2. Kaplan-Meier analysis curve of postoperative survival times of lung cancer patients according to the miRNA-31 relative expression. Patients with high miRNA-31 expression had significantly poorer survival times compared to those with low miRNA-31 expression. P values were calculated by Kaplan-Meier analysis (P<0.001).

development, and prognosis have been widely studied with the development of miRNA expression spectrum research [12–16]. The initial evidence of the relationship between miRNA and tumor was from the study of Calin et al. in 2002 [17], who found that expression of miR-15a and miR-16 are decreased or deleted in most chronic lymphocytic leukemia patients. miRNA stability is a prerequisite for potential tumor markers [18]. A recent meta-analysis indicates that miR-21 detection has a prognostic value in patients with gastric cancer [19]. Another recent study suggested that the expression of miR-101 is down-regulated in bladder transitional cell carcinoma (BTCC) and may play an important role as a diagnostic and prognostic marker in BTCC [20]. Utilizing miRNA expression level in peripheral blood to diagnose tumors early is effective and deserves to be explored further because miRNA is very stable in blood plasma and serum. Liu et al. [8] found that miRNA-31 is highly expressed in lung cancer tissue. Xi et al. [9] confirmed that miRNA-31 expression in non-small-cell lung carcinoma tissue was 4.13-fold that in normal lung tissue. We have detected the expression of miRNA-31 in peripheral blood of 300 cases of primary lung cancer and 300 healthy adults. Our results show that miRNA-31 expression level in peripheral blood of lung cancer patients is significantly higher than that of health adults. miRNA-31 expression level in peripheral blood of lung cancer patients who are smokers with high clinical stage and lymph node metastasis are significantly higher than that of patients who are not smokers and who have low clinical stage and are without lymph node metastasis.

MiRNA-31 was first identified in HeLa cells [21] and is located on chromosome 9p21.3. Mounting evidence shows that MiRNA-31 has different expression patterns in different cancers: it is up-regulated in colorectal cancer (CRC) [22], head and neck squamous cell carcinoma (HNSCC) [23], hepatocellular carcinoma [24], squamous cell carcinoma of the tongue [25], and lung cancer [26]; but it is down-regulated in invasive urothelial carcinoma of the bladder [27], prostate cancer [28], gastric cancer [29], breast cancer [30], and serous ovarian cancer [31]. Although there is growing evidence that miR-31 level varies among cancer types, functional roles for miR-31 have yet to be defined. However, the circulating miR-31 level may act as a clinical biomarker for cancer. In the present study, we drew the ROC curve according to miRNA-31 expression level in peripheral blood, and the area under curve reached 0.785 (95% CI=0.486–0.763). Survival analysis shows that median survival period of the miRNA low-expression group was longer than that of the miRNA high-expression group, suggesting that miRNA-31 expression level in peripheral blood has good diagnostic value and can be utilized to evaluate patient prognosis.

Conclusions

Our study has confirmed the existence of miRNA-31 in peripheral blood of lung cancer patients, showing that the expression level of miRNA-31 in peripheral blood can be utilized in clinics as a molecular marker for evaluation of lung cancer diagnosis and prognosis.

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