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MiR-520f acts as a biomarker for the diagnosis of lung cancer

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Abstract

Lung cancer is a malignant tumor with high morbidity and mortality. Early diagnosis remains a great challenge for the cancer. In this study, we aimed to explore diagnostic performance of serum microRNA-520f (*miR-520f*) in lung cancer.

Serum specimens were collected from 139 lung cancer patients and 76 healthy volunteers. Relative expression level of serum *miR-520f* was detected adopting quantitative real-time polymerase chain reaction (qRT-PCR). Chi-square test was applied to evaluate the association of *miR-520f* with clinical parameters of the patients. Additionally, receiver operating characteristic (ROC) analysis was performed to evaluate diagnostic value of *miR-520f* in lung cancer.

Serum *miR-520f* was down-regulated in lung cancer patients compared with healthy group ($P < .001$). Moreover, the expression of *miR-520f* was significantly associated with advanced TNM stage ($P = .031$) and metastasis ($P = .002$). The area under the curve (AUC) value of ROC curve was 0.888, suggesting that *miR-520f* could be a diagnostic biomarker for lung cancer. The cut-off value of serum *miR-520f* for lung cancer diagnosis was 1.815, with a sensitivity of 79.9% and a specificity of 84.2%.

Serum *miR-520f* was down-regulated in lung cancer patients, and may be a candidate biomarker for non-invasive screening of the disease.

Abbreviations: AUC = area under the curve, CT = computer tomography, miR-520f = microRNA-520f, NSCLC = non-small cell lung cancer, qRT-PCR = quantitative real-time polymerase chain reaction, ROC = receiver operating characteristic.

Keywords: diagnosis, lung cancer, miR-520f

1. Introduction

Lung cancer is one of the most common cancers, representing a leading cause for cancer-related deaths worldwide.[1] In China, the morbidity of lung cancer is on the rise, due to aging, pollution, and exposure to carcinogens.[2,3] Lung cancer includes 2 pathological types: non-small cell lung cancer (NSCLC) and small cell lung cancer. NSCLC is the major type, accounting for more than 80% of whole lung cancer cases.[4] Great progress has been made in the treatments of lung cancer, but long-term survival of the patients does not show significant improvements.[5,6] Delayed diagnosis may be responsible for dismal prognosis.[7] The cancer is characterized by silent growth at early stages, and most of the lung cancer patients present distant invasion or metastasis when initially diagnosed, leading to limited therapeutic effects and poor clinical outcomes.[8,9] Therefore, finding effective biomarkers for early diagnosis and treatment would be a promising approach to improve the prognosis of lung cancer patients.

MicroRNAs (miRNAs) are a group of small non-coding RNAs that play important roles in gene expression at post-transcriptional level.[10] MiRNAs are involved in a variety of biological processes, such as cell proliferation, differentiation, development, and apoptosis.[11] Abnormal expression of miRNAs may lead to various pathological states, like cancer. Expression profiles of miRNAs show close association with the development and progression of as well as treatment response to cancers, suggesting their abilities to serve as predictive biomarkers and therapeutic targets for diseases.[12] In addition, expression patterns of miRNAs are stable in body fluids and archived specimens, thus being detected through non or minimally invasive methods.[13] Circulating miRNAs are considered as promising biomarkers for early screening and supervision of cancers.

MicroRNA-520f (miR-520f), a common member of miRNA family, was reported to play important roles in the progression of human cancers. For instance, *miR-520f* knockdown in gastric cancer cells in vitro could lead to aggressive cellular proliferation, revealing its suppressive effects against the development and progression of the cancer.[14] Animal studies have proved that *miR-520f* over-expression could inhibit lung metastasis in mouse model.[15] However, diagnostic performance of serum *miR-520f* in lung cancer remained unclear.

In our study, we aimed to detect expression pattern of serum *miR-520f* in lung cancer patients, as well as its association with clinical parameters of the cases. In addition, receiver operating characteristic (ROC) curve was constituted to estimate diagnostic value of serum *miR-520f* in lung cancer.

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2. Materials and methods

2.1. Patients and specimen collection

A total of 139 lung cancer patients who were diagnosed by pathologists between October 2015 and November 2017 were finally recruited from Ningbo Medical Center Lihuili Hospital. None of the patients had received any treatments in advance. Additionally, 76 healthy volunteers who were matched with the cases in gender and age were collected as the control group. In the healthy group, no one had been diagnosed with any malignancies. This study was approved by the ethics committee of the hospital. Informed consents were also obtained from all patients and healthy individuals. Clinicopathological features of the lung cancer patients were listed in Table 1, including age, gender, tumor size, differentiation, pathologic types, TNM stage, and metastasis.

A total of 5 mL fasting blood samples was taken from each of 139 lung cancer patients and 76 healthy volunteers. Then serum specimens were isolated from the blood through centrifugation and stored at −80°C until RNA extraction.

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

Total RNA was isolated from serum specimens using Trizol (Invitrogen). Then the first-strand cDNA was synthesized employing TaqMan MicroRNA Reverse Transcription Kit (Applied biosystems, Carlsbad, CA). In this study relative level of serum, *miR-520f* was determined using the SYBR Green Realtime PCR Master Mix (QPK-201, Toyobo Co, Ltd, Osaka, Japan). *U6* acted as an internal control. Relative expression level of *miR-520f* was normalized to *U6* and calculated using the $2^{-\Delta\Delta Ct}$ method.

Table 1

Association of *miR-520f* expression with clinicopathological parameters of lung cancer patients.

Characteristics	No. N = 139	*MiR-520f* expression		*P* values
		High (n = 64)	Low (n = 75)	
Age (yr)				
<60	62	30	32	.619
≧60	77	34	43	
Gender				
Male	69	34	35	.448
Female	70	30	40	
Tumor size				
<3cm	71	31	40	.565
≧3cm	68	33	35	
Differentiation				
Well	74	33	41	.715
Moderate-poor	65	31	34	
Pathologic types				
SCLC	58	27	31	.919
NSCLC	81	37	44	
TNM stage				
I-II	82	44	38	.031
III-IV	57	20	37	
Metastasis				
Yes	59	18	41	.002
No	80	46	34	

NSCLC = non-small cell lung cancer.

2.3. Statistical analysis

All statistical analyses were performed with SPSS 19.0 (SPSS Inc., Chicago, IL) and GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA). Expression level of *miR-520f* was expressed as mean ± SD. Student *t* tests was used to analyze differences in *miR-520f* expression levels between the case and control groups. The relationship between *miR-520f* expression and various clinicopathological characteristics was assessed using Chi-square tests. To determine diagnostic performance of *miR-520f* expression in lung cancer, we performed ROC (receiver operating characteristic) analysis. The sensitivity and misjudgment rate (1-Specificity) can be obtained through the movement of cut off point/cut off value using SPSS 19.0 to draw ROC curve. With sensitivity as the vertical axis and misjudgment rate as the horizontal axis, the curve can be drawn via connecting the points. Then the area under the curve (AUC) an be calculated. The larger the area appears, the higher the judgment value is. The best cut off value is commonly used to determine the "Youden Index". Maximum value of the index is the best boundary value. $P < .05$ in this study was considered statistically significant.

3. Results

3.1. Down-regulated expression of miR-520f in lung cancer

To examine *miR-520f* expression in 139 lung cancer patients and 76 healthy controls, qRT-PCR was performed. The results shown in Figure 1 revealed that the expression of *miR-520f* was significantly lower in lung cancer patients than in healthy volunteers ($P < .001$).

3.2. Association between miR-520f and clinicopathological parameters of lung cancer patients

In this study, the lung cancer cases were categorized into high expression group (n = 64) and low expression group (n = 75), according to their average expression value of *miR-520f*. Chi-square test was used to detect the correlation between *miR-520f*

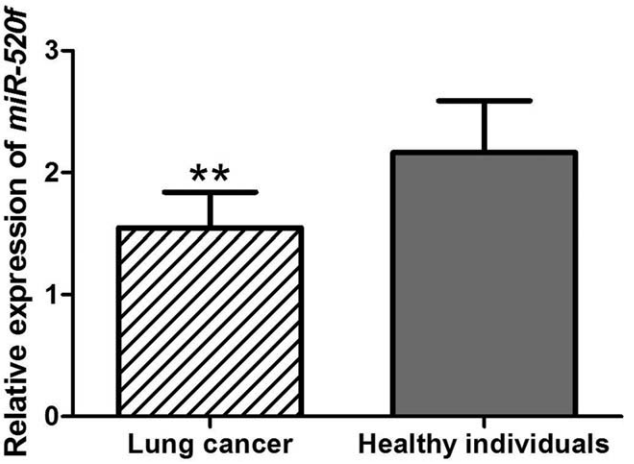

Figure 1. Serum *miR-520f* expression levels evaluated by qRT-PCR in lung cancer patients and healthy controls. Analysis results demonstrated that lung cancer patients exhibited significantly up-regulated *miR-520f* level, compared with healthy individuals. **: $P < .01$. qRT-PCR = quantitative real-time polymerase chain reaction.

expression level and clinicopathological data of lung cancer patients. *MiR-520f* expression was negatively associated with TNM stage ($P=.031$) and metastasis ($P=.002$). However, expression pattern of *miR-520f* did not show significant association with age, gender, differentiation or pathologic types among the patients with lung cancer (all $P>.05$) (Table 1).

3.3. Diagnostic value of miR-520f expression in lung cancer

ROC curves were plotted based on serum levels of *miR-520f* in lung cancer cases and healthy individuals in our study. The curve showed that lung cancer patients could be distinguished from healthy volunteers according to their serum levels of *miR-520f* with an AUC value of 0.888. The cut-off value for *miR-520f* diagnosing lung cancer was 1.815, with a sensitivity of 79.9% and a specificity of 84.2% (Fig. 2).

4. Discussion

Lung cancer is a fatal cancer with high morbidity and mortality.[16] Low diagnosis rate may be responsible for dismal prognosis of the cancer.[17] Until now, early screening of lung cancer mainly depends on computer tomography (CT)-based strategies. However, those approaches are high cost and invasive, and their accuracy is limited by tumor type and size.[18,19] Therefore, reliable biomarkers which can be employed as minimally invasive tools for early detection of lung cancer are in urgent need in clinic. In this study, we investigated diagnostic performance of serum *miR-520f* in lung cancer. ROC analysis suggested that serum *miR-520f* might be a diagnostic biomarker for lung cancer.

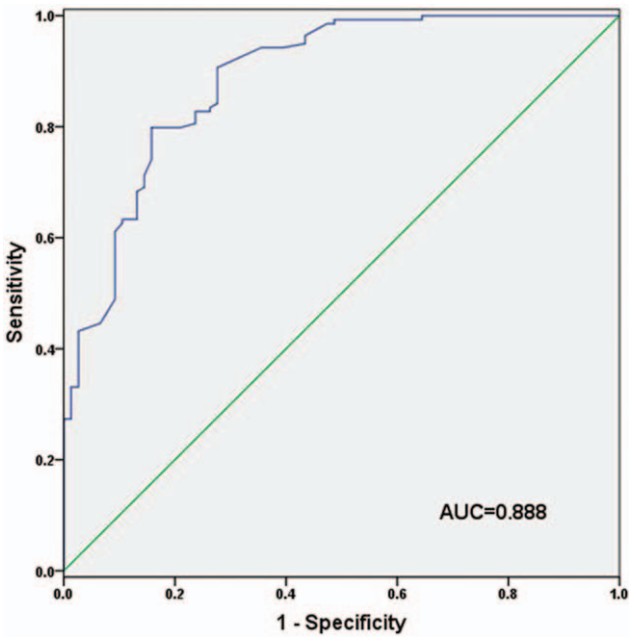

Figure 2. ROC analysis evaluating diagnostic accuracy of serum *miR-520f* in lung cancer. The curve suggested that serum *miR-520f* could discriminate between lung cancer cases and healthy volunteers with an AUC value of 0.888, combing with a sensitivity of 79.9% and a specificity of 84.2%. AUC = area under the curve, ROC = receiver operating characteristic.

MiRNAs are a group of small endogenous RNA molecules that can mediate gene expression by binding to the 3' untranslated region (3' UTR) of their target genes.[20] MiRNAs as oncogenes or tumor suppressors play an important role in tumorigenesis, and their expression profiles show specific to the development and progression of malignancies.[21] Yu et al reported that plasma levels of *miR-92a-2* were significantly different between small cell lung cancer patients and healthy individuals, suggesting its function as a biomarker for early detection of the disease.[22] The study carried out by Shi et al reported that serum levels of *miR-22* and *miR-15b* could be employed as reference factors for early detection of NSCLC, thus enhancing diagnostic accuracy of serum CEA.[23] MiRNAs might be used for prognosis evaluation as well. The meta-analysis constructed by Zhan et al demonstrated that expression profiles of *miR-21*, *miR-200c*, *miR-125b*, *miR-148b*, *miR-365*, *miR-124*, *miR-32*, *miR-146a*, and *miR-375* showed close association with clinical outcomes of NSCLC patients, revealing their abilities as prognostic biomarkers for the cancer.[24] MiRNAs may be promising biomarkers for early screening and prognosis evaluation in human malignancies.

In our study, we investigated the function of *miR-520f* in lung cancer. Serum level of *miR-520f* mRNA was significantly down-regulated in lung cancer cases, compared with healthy volunteers. Moreover, its expression level was negatively correlated with TNM stage and metastasis. Patients with low serum level of *miR-520f* were more likely to present advanced TNM stage and positive metastasis. The data might reveal that *miR-520f* acted as a tumor suppressor gene, and that its inhibition could promote the development and progression of lung cancer. In vitro studies have demonstrated that miR-520 could regulate invasive and metastatic abilities of cancer cells. Moreover, the down-regulating of *miR-520f* might contribute to drug resistance via weakening apoptosis inhibition.[15,25] Taken together, *miR-520f* might be a tumor suppressor gene in malignancies. Studies indicated that *miR-520f* inhibited the proliferation and invasion of hepatocellular carcinoma cells through targeting TM4SF1.[26]*MiR-520f* also suppressed the invasion of cancer cells via targeting ADAM9 and TGFBR2, had the anti-invasion and anti-metastasis effects in vitro and in vivo.[27] All these findings may provide therapeutic approaches for cancer treatment.

Consequently, we investigated diagnostic value of serum *miR-520f* in lung cancer. The results showed that the expression of *miR-520f* could distinguish lung cancer patients from healthy individuals with high sensitivity and specificity. Serum *miR-520f* might be a non-invasive biomarker in early detection of lung cancer. However, there were still several limitations in the present study. First, we proved that *miR-520f* was down-regulated in lung cancer, but its specific molecular mechanism is still unknown. Second, our results might be limited by the relatively small sample size. Well-designed researches with larger sample sizes are required to verify diagnostic significance of serum *miR-520f* in lung cancer.

In conclusion, serum *miR-520f* is downregulated in lung cancer, and its expression level shows negative association with advanced tumor stage and metastasis. Serum *miR-520f* may be a biomarker in early detection of lung cancer.

Author contributions

Conceptualization: Yingyan Zhou.
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Supervision: Shimo Shen.
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Visualization: Shimo Shen.
Writing – original draft: Shimo Shen.
Writing – review & editing: Shimo Shen.

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