European Review for Medical and Pharmacological Sciences 2017; 21: 4092-4097

Investigation of serum miR-411 as a diagnosis and prognosis biomarker for non-small cell lung cancer

S.-Y. WANG[1], Y. LI[1], Y.-S. JIANG[1], R.-Z. LI[2]

[1]Department of Respiratory Medicine, The Second People's Hospital of Liaocheng, Liaocheng, Shandong Province, China
[2]Department of Medical Laboratory Medicine, Jining No. 1 People's Hospital, Jining, Shandong Province, China

Abstract. – OBJECTIVE: Biomarkers in blood have become increasingly appreciated in the diagnosis and prognosis of non-small cell lung cancer (NSCLC). The purpose of the current study was to explore potential diagnostic and prognostic value of serum miR-411 in NSCLC patients.

PATIENTS AND METHODS: 153 patients with NSCLC and 75 healthy controls were enrolled in the study. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was performed to determine the expression level of serum miR-411 in NSCLC patients and healthy controls. The association of miR-411 expression with clinicopathological factors or the prognosis of NSCLC patients was also analyzed. Patient survival was determined by telephone interview, and survival curves were plotted by using the Kaplan-Meier method and compared by the Log-rank test. Receiver operating characteristic curves was used to evaluate the sensitivity and specificity of serum miR-411.

RESULTS: Our data showed that serum miR-411 levels were significantly greater in NSCLC patients than healthy controls ($p < 0.001$). High serum miR-411 expression was significantly associated with TNM stage ($p = 0.018$), lymph node status ($p = 0.006$) and differentiated degree ($p < 0.015$). Kaplan-Meier analysis showed that high serum miR-411 expression levels predicted poor survival ($p = 0.0053$). The area under the curve (AUC) of high expression of serum miR-411 to diagnose NSCLC was 0.835 (95% CI: 0.737-0.933, $p < 0.001$). Univariate and multivariate analyses suggested that high expression of miR-411 was an independent poor prognostic indicator for NSCLC patients.

CONCLUSIONS: The present results suggested that detection of serum miR-411 levels may have clinical potentials as a non-invasive diagnostic/prognostic biomarker for NSCLC patients.

Key Words:
Serum miR-411, NSCLC, Prognosis, Diagnosis.

Introduction

Lung cancer remains the leading cause of cancer deaths among both men and women with a five-year survival rate of 18.7%[1]. In recent years, lung cancer has been increasing rapidly in China[2]. Non-small cell lung cancer (NSCLC) is the most common histological subtype, accounting for 80-85% of all lung cancers[3]. Despite recent advances in diagnostic and therapeutic procedures, about 30% of these NSCLC patients showed recurrence or metastasis during five years period[4,5]. It was reported that the 5-year survival rate could reach to 50% if it was early detected and received treatment[6]. Currently, the widely used diagnostic markers for NSCLC are CEA, CYFRA21-1 and SCC[7]. However, these tumor markers often fail to improve the rate of early diagnosis, which results in NSCLC spreading. Thus, it is necessary to search for novel markers for NSCLC, which can accurately identify the biological characteristics of tumors and help clinical treatments. MicroRNAs (miRNAs), approximately 18-25 nucleotides in length, are a group of endogenous small and noncoding RNAs[8]. It has been well known that miRNAs play important roles in a variety of biological processes, such as development, differentiation, proliferation, and apoptosis[9]. Recent studies have shown that miRNAs play critical roles in the development, invasion and metastasis of tumors[10], including NSCLC. For instance, Yao et al[11] reported that miR-325-3p upregulation inhibited cell

Corresponding Author: Ren-Zhe Li, MD; e-mail: lrz030609@163.com

invasion and proliferation by targeting HMGB1 in NSCLC. Zhang et al[12] reported that miR-377 overexpression reduced NSCLC cell proliferation and promoted apoptosis by targeting CDK6. These findings indicated that miRNAs play different roles in tumors. Indeed, growing evidence showed that miRNAs can function either as tumor suppressors or as oncogenes[13,14]. The important role of miRNAs in the development of tumors highlights the potential as diagnostic and prognostic biomarkers in cancers[15]. Zhao et al[16] showed that miR-411 was overexpressed in the lung cancer cells, and it served as a tumor promoter. However, the detail role of miR-411 in NSCLC remains largely unknown. In the present study, we aimed to study whether serum miR-411 could represent a predictive biomarker for the prognosis and diagnosis of NSCLC patients.

Patients and Methods

Patient Specimens

Between July 2010 and June 2012, 153 patients with NSCLC were admitted to the Second People's Hospital of Liaocheng and Underwent Surgical Resection. The diagnosis of all cases of NSCLC was confirmed histologically, based mainly on examination of sections stained with hematoxylin and eosin (H&E). The 153 NSCLC patients consisted of 96 males and 57 females, and the age ranged from 37 to 77 years. None of the patients had received any other therapy, including percutaneous ablation and chemo-embolization before surgery. All the NSCLC patients were followed for 60 months and a set of complete clinical data was recorded properly. In the control group, 75 blood samples were collected from individuals who had previously been diagnosed without any type of tumors. The detail clinical information was shown in Table II. Written informed consent was obtained from all patients, and the study was approved by the Ethics Committee of The Second People's Hospital of Liaocheng.

RNA Extraction and RT-PCR

To obtain serum samples, 10 mL of peripheral blood was drawn into separate gel tubes and then subjected within 30 min to centrifugation at 1.500 g for 10 min at 4°C. The supernatants were stored at -80°C until use. Total RNA was extracted from the serum using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. Then, the RNA was reverse transcribed using SuperScript First Strand cDNA System (Thermo Fisher Scientific, Waltham, MA, USA). The miR-411 expression was measured by qRT-PCR using an ABI7300HT instrument (Applied Biosystems, Foster City, CA, USA). Reactions were incubated in a 96-well optical plate at 95°C for 10 min, followed by 45 cycles of 95°C 15 s, 60°C for 60 s. Results were normalized to the expression of U6. The relative gene expression was calculated using $2^{-\Delta\Delta Ct}$ method. Primers used in qRT-PCR were shown in Table I.

Statistical Analysis

All the statistical analyses and graphics were performed with GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA) or SPSS version13.0 for Windows (SPSS Inc, Chicago, IL, USA). The X^2 and *t*-tests were performed to explore the associations between the miR-411 expression level and the clinical characteristics. Overall survival curves were plotted according to the Kaplan-Meier method, and the log-rank test was applied for comparison. Cox's proportional hazards model was used to identify the factors that have significant influence on survival. Diagnostic accuracy of serum miR-411 was assessed employing receiver operating characteristic (ROC) curve. $p < 0.05$ was considered statistically significant.

Results

Evaluation of Serum miR-411 Expression Level in NSCLC Patients and Healthy Volunteers

We first checked the expression status of miR-411 the serum of the NSCLC patients. As shown in Figure 1, our results indicated that serum miR-411 levels were significantly greater in NSCLC patients than healthy controls ($p < 0.001$). These results indicated that miR-414 might play an oncogenic role in NSCLC.

Association Between Serum miR-411 Expression and Clinical Pathological Features

To evaluate the potential relationship between serum miR-411 expression and NSCLC clinicopathologic features, we divided the samples into high (above the median, n= 78) and low (below the median, n=75) serum miR-411 expression groups according to the median value of serum miR-411 levels. Noticeably, high serum miR-

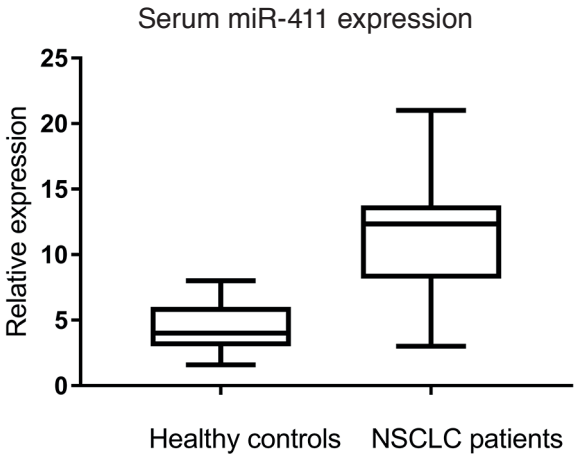

Figure 1. The expression of serum miR-411 was measured in NSCLC patients and healthy controls by qRT-PCR. Serum miR-411 expression was remarkably increased in NSCLC patients relative to that in healthy controls ($p < 0.001$).

411 expression was significantly associated with TNM stage ($p = 0.018$), lymph node status ($p = 0.006$) and differentiated degree ($p < 0.015$). However, we did not find a significant association of serum miR-411 expression levels with age, gender, smoking history and distant metastasis ($p > 0.05$, respectively).

Correlation Between Serum Expression of miR-411 and Survival Time of NSCLC Patients

We further evaluated the association of serum miR-411 expression level with the survival of NSCLC patients. The Kaplan-Meier curve revealed that the upregulation level of serum miR-411 was related to worse overall survival ($p = 0.0053$, Figure 2). We further performed a Cox multivariate analysis to identify independent prognostic markers for NSCLC. Univariate analysis of factors related to overall survival in NSCLC is summarized in Table III. TNM stage, lymph node status, differentiated degree and serum miR-411 expression were significant prognostic factors for

Table I. The primer sequence of serum miR-411 and U6.

Gene	Sequence
miR-411	F: 5'-GGGGTAGTAGACCGTATAG-3' R: 5'-TGCGTGTCGTGGAGTC-3'
U6	F: 5′-GTTGCGTTACACCCTTTCTTG-3′ R: 5′-GTCACCTTCACCGTTCCAGT-3′

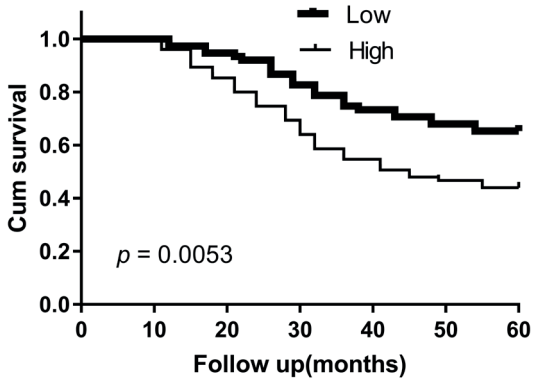

Figure 2. Kaplan-Meier survival curves of patients with NSCLC based on serum miR-411 expression status. Patients with high serum miR-411 expression had a shorter overall survival than those low expression ($p = 0.0053$).

overall survival of NSCLC patients. Furthermore, in a multivariate Cox model, we found that serum miR-411 expression was an independent poor prognostic factor for overall survival in NSCLC patients (HR=3.789, $p = 0.008$, Table III).

Diagnostic Accuracy of Serum miR-411 for NSCLC

To investigate the characteristics of serum miR-411 as potential tumor markers of NSCLC, we performed the ROC curve and the area under the ROC curves (AUC) on NSCLC patient and healthy controls. As shown in Figure 3, our results suggested that the area under the curve (AUC) of high expression of serum miR-411 to diagnose NSCLC, was 0.835 (95% CI: 0.737-0.933, $p < 0.001$). These results showed that the relative expression of serum miR-411 level could distinguish NSCLC patients from healthy controls.

Discussion

The development of a tumor is a multi-stage slow process, involving a wide range of molecular biological changes[17]. More and more tumor markers have been found to be abnormally expressed in tissues and serum of patients. Previous studies indicated that some markers could be used for potential biomarkers for the diagnosis and prognosis of NSCLC like serum protease activated receptor1[18], glycosylated alpha-1-acid glycoprotein 1[19] and serum HE4[20]. Since circulating miRNAs were discovered in 2008[21], they rapidly became the hot point for the diagnosis and prognosis of human can-

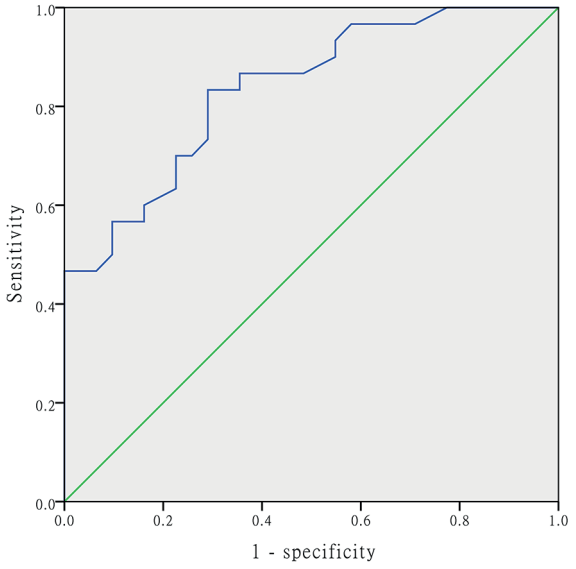

Figure 3. Diagnostic value of serum miR-411 expression for NSCLC patients. ROC curve analysis illustrated that serum miR-411 expression was a potential biomarker for screening NSCLC patients from healthy controls (AUC = 0.835, 95% CI: 0.737-0.933, $p < 0.001$).

cers[22]. Some serum miRNAs have been reported to be potential diagnostic and prognostic biomarkers for tumor, such as serum miR-205 for cervical cancer[23], serum miR-224 for hepatocellular carcinoma[24] and serum miR-497 for osteosarcoma[25]. MiR-411 was encoded at 14q32 and belonged to the 14q32.31 miR cluster B[26]. Previous studies have reported that miR-411 may play a positive or negative role in tumorigenesis. For instance, Guo et al[27] reported that miR-411 was significantly downregulated in breast cancer, and over-expression of miR-411 significantly suppressed breast cancer cell growth, migration, and invasion, by targeting specificity protein 1. Xia et al[28] found that ectopic expression of miR-411 promoted the proliferation of human hepatocellular carcinoma cells by targeting ITCH expression. The above results suggested that miR-411 served as a tumor suppressor in hepatocellular carcinoma and breast cancer. Most recently, Nadal et al[26] reported that miR-411 expression was significantly up-regulated in lung cancer. By survival analysis, they found that patients with higher miR-411 expression level are associated with a poorer overall survival. Also, another study by Zhao et al[16] showed that overexpression of miR-411 promoted cell proliferation of lung cancer by targeting tumor suppressor gene FOXO1. These results indicated that miR-411 served as a tumor promoter in NSCLC. However, up to date, although the prognostic value of miR-411 has been reported, the diagnostic value of serum miR-411 in NSCLC has not been repor-

Table II. Association of serum miR-411 expression with clinicopathological variables in 153 NSCLC patients.

Characteristic	Case number	Serum miR-411 expression		*p*
		Low	High	
Age (years)				0.389
<60	64	34	30	
≥60	89	41	48	
Gender				0.491
Male	96	45	51	
Female	57	30	27	
Smoking history				0.344
Yes	90	47	43	
No	63	28	35	
TNM stage				0.018
I+II	100	56	44	
III+IV	53	19	34	
Lymph node status				0.006
Yes	42	13	29	
No	111	62	49	
Distant metastasis				0.125
Yes	52	21	31	
No	101	54	47	
Differentiated degree				0.015
Low/middle	40	13	27	
High	113	62	51	

Table III. Cox regression analysis of factors associated with overall survival in 153 NSCLC patients.

	Univariate analysis		Multivariate analysis	
Variable	HR	*p*-value	HR	*p*-value
Age	1.321	0.421	-	-
Gender	1.289	0.266	-	-
Smoking history	1.533	0.179	-	-
TNM stage	3.442	0.011	2.782	0.016
Lymph node status	3.852	0.006	3.231	0.009
Distant metastasis	1.782	0.079	-	-
Differentiated degree	3.231	0.009	2.663	0.013
Serum miR-411	4.519	0.004	3.789	0.008

ted. In the present study, we firstly detected the expression levels of serum miR-411 in NSCLC patients. Our results showed that serum miR-411 levels were significantly higher in NSCLC patients than healthy controls. Next, we analyzed the association between the serum miR-411 expression and various clinicopathological factors and found that high serum miR-411 expression was significantly associated with TNM stage, lymph node status, and differentiated degree. Moreover, the results of Kaplan-Meier method showed that the overall survival time of patients with higher serum miR-411 expression levels was shorter than that of patients with lower serum miR-411 expression levels. In a multivariate Cox model, our results suggested that serum miR-411 expression level was independent prognostic factors for overall survival of NSCLC patients. In order to further explore the diagnostic value of serum miR-411 in NSCLC patients, we performed ROC analysis. For a high AUC value, sensitivity and specificity on the basis of ROC analysis, the diagnostic value of serum miR-411 was considered to be valuable.

Conclusions

Our findings, for the first time, provided convincing evidence that elevated serum miR-411 expression represented high diagnostic and prognostic value, suggesting that serum miR-411 might be a useful diagnostic and prognostic marker for NSCLC.

Conflict of interest

The authors declare no conflicts of interest.

References

1) Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010; 127: 2893-2917.
2) Chen G, Sun X, Ren H, Wan X, Huang H, Ma X, Ning B, Zou X, Hu W, Yang G. The mortality patterns of lung cancer between 1990 and 2013 in Xuanwei, China. Lung Cancer 2015; 90: 155-160.
3) Spira A, Ettinger DS. Multidisciplinary management of lung cancer. N Engl J Med 2004; 350: 379-392.
4) Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010; 60: 277-300.
5) Margaritora S, Cesario A, Cusumano G, Dall'armi V, Porziella V, Meacci E, Lococo F, D'Angelillo R, Congedo MT, Granone P. Pneumonectomy with and without induction chemo-radiotherapy for non-small cell lung cancer: short and long-term results from a single centre. Eur Rev Med Pharmacol Sci 2013; 17: 29-40.
6) Kowalczuk O, Burzykowski T, Niklinska WE, Kozlowski M, Chyczewski L, Niklinski J. CXCL5 as a potential novel prognostic factor in early stage non-small cell lung cancer: results of a study of expression levels of 23 genes. Tumour Biol 2014; 35: 4619-4628.
7) Sulpher JA, Owen SP, Hon H, Tobros K, Shepherd FA, Sabri E, Gomes M, Sekhon H, Liu G, Canil CM, Wheatley-Price P. Factors influencing a specific pathologic diagnosis of non-small-cell lung carcinoma. Clin Lung Cancer 2013; 14: 238-244.
8) Pipan V, Zorc M, Kunej T. MicroRNA polymorphisms in cancer: a literature analysis. Cancers (Basel) 2015; 7: 1806-1814.
9) Macfarlane LA, Murphy PR. MicroRNA: biogenesis, function and role in cancer. Curr Genomics 2010; 11: 537-561.
10) Nicoloso MS, Spizzo R, Shimizu M, Rossi S, Calin GA. MicroRNAs--the micro steering wheel of tumour metastases. Nat Rev Cancer 2009; 9: 293-302.
11) Yao S, Zhao T, Jin H. Expression of MicroRNA-325-3p and its potential functions by targeting HMGB1 in non-small cell lung cancer. Biomed Pharmacother 2015; 70: 72-79.

12) Zhang J, Zhao M, Xue ZQ, Liu Y, Wang YX. miR-377 inhibited tumorous behaviors of non-small cell lung cancer through directly targeting CDK6. Eur Rev Med Pharmacol Sci 2016; 20: 4494-4499.

13) Jin Z, Guan L, Song Y, Xiang GM, Chen SX, Gao B. MicroRNA-138 regulates chemoresistance in human non-small cell lung cancer via epithelial mesenchymal transition. Eur Rev Med Pharmacol Sci 2016; 20: 1080-1086.

14) Li YL, Wang J, Zhang CY, Shen YQ, Wang HM, Ding L, Gu YC, Lou JT, Zhao XT, Ma ZL, Jin YX. MiR-146a-5p inhibits cell proliferation and cell cycle progression in NSCLC cell lines by targeting CCND1 and CCND2. Oncotarget 2016; 7: 59287-59298.

15) Hennessey PT, Sanford T, Choudhary A, Mydlarz WW, Brown D, Adai AT, Ochs MF, Ahrendt SA, Mambo E, Califano JA. Serum microRNA biomarkers for detection of non-small cell lung cancer. PLoS One 2012; 7: e32307.

16) Zhao Z, Qin L, Li S. miR-411 contributes the cell proliferation of lung cancer by targeting FOXO1. Tumour Biol 2016; 37: 5551-5560.

17) Lesko E, Majka M. The biological role of HGF-MET axis in tumor growth and development of metastasis. Front Biosci 2008; 13: 1271-1280.

18) Erturk K, Tastekin D, Bilgin E, Tas F, Disci R, Duranyildiz D. Clinical significance of serum protease activated receptor1 levels in patients with lung cancer. Eur Rev Med Pharmacol Sci 2016; 20: 243-249.

19) Ayyub A, Saleem M, Fatima I, Tariq A, Hashmi N, Musharraf SG. Glycosylated alpha-1-acid glycoprotein 1 as a potential lung cancer serum biomarker. Int J Biochem Cell Biol 2016; 70: 68-75.

20) Iwahori K, Suzuki H, Kishi Y, Fujii Y, Uehara R, Okamoto N, Kobayashi M, Hirashima T, Kawase I, Naka T. Serum HE4 as a diagnostic and prognostic marker for lung cancer. Tumour Biol 2012; 33: 1141-1149.

21) Lawrie CH, Gal S, Dunlop HM, Pushkaran B, Liggins AP, Pulford K, Banham AH, Pezzella F, Boultwood J, Wainscoat JS, Hatton CS, Harris AL. Detection of elevated levels of tumour-associated microRNAs in serum of patients with diffuse large B-cell lymphoma. Br J Haematol 2008; 141: 672-675.

22) Zhu W, Zhou K, Zha Y, Chen D, He J, Ma H, Liu X, Le H, Zhang Y. Diagnostic value of serum miR-182, miR-183, miR-210, and miR-126 levels in patients with early-stage non-small cell lung cancer. PLoS One 2016; 11: e0153046.

23) Ma Q, Wan G, Wang S, Yang W, Zhang J, Yao X. Serum microRNA-205 as a novel biomarker for cervical cancer patients. Cancer Cell Int 2014; 14: 81.

24) Lin L, Lu B, Yu J, Liu W, Zhou A. Serum miR-224 as a biomarker for detection of hepatocellular carcinoma at early stage. Clin Res Hepatol Gastroenterol 2016; 40: 397-404.

25) Pang PC, Shi XY, Huang WL, Sun K. miR-497 as a potential serum biomarker for the diagnosis and prognosis of osteosarcoma. Eur Rev Med Pharmacol Sci 2016; 20: 3765-3769.

26) Nadal E, Zhong J, Lin J, Reddy RM, Ramnath N, Orringer MB, Chang AC, Beer DG, Chen G. A MicroRNA cluster at 14q32 drives aggressive lung adenocarcinoma. Clin Cancer Res 2014; 20: 3107-3117.

27) Guo L, Yuan J, Xie N, Wu H, Chen W, Song S, Wang X. miRNA-411 acts as a potential tumor suppressor miRNA via the downregulation of specificity protein 1 in breast cancer. Mol Med Rep 2016; 14: 2975-2982.

28) Xia K, Zhang Y, Cao S, Wu Y, Guo W, Yuan W, Zhang S. miR-411 regulated ITCH expression and promoted cell proliferation in human hepatocellular carcinoma cells. Biomed Pharmacother 2015; 70: 158-163.