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Evaluating the diagnostic and prognostic value of serum miR-770 in non-small cell lung cancer

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Abstract. – OBJECTIVE: Serum miRNAs are promising biomarkers for the diagnosis and prognostication of many diseases. Our present study aimed at assessing the usefulness of serum miR-770 expression as a diagnostic and prognostic tool for non-small-cell lung cancer (NSCLC).

PATIENTS AND METHODS: Quantitative Real-time PCR (qRT-PCR) was used to measure expression level of serum miR-770 in 196 NSCLC patients and 77 healthy controls. The associations of serum miR-770 expression with clinicopathologic variables were analyzed. Receiver operating characteristic analysis (ROC) was used to determine the value of serum miR-770 as a biomarker for diagnosis of NSCLC. Kaplan-Meier and Cox regression analyses were performed to examine the relationship between serum miR-770 expression and prognosis of NSCLC patients.

RESULTS: We found that serum miR-770 levels were significantly decreased in NSCLC patients compared to healthy controls ($p < 0.01$). Decreasing serum miR-770 expression was significantly correlated with poorer histological grade ($p = 0.015$), positive lymph node metastasis ($p = 0.007$), and advanced tumor stage ($p = 0.002$). Moreover, we found that the diagnostic value of serum miR-770 for NSCLC patients from healthy controls resulted in an AUC of 0.835 (95 % CI, 0.751-0.919) with a sensitivity of 68% and a specificity of 89%, based on a cut-off value of 5.723. Kaplan-Meier analysis showed that decreased serum miR-770 expression was associated with poor overall survival of NSCLC patients ($p = 0.000$). Multivariate analysis confirmed that serum miR-770 was an independent prognostic factor in patients with NSCLC.

CONCLUSIONS: Our findings suggested that serum miR-770 may represent a novel diagnostic, prognostic biomarker and a potential therapeutic target for NSCLC.

Key Words:
Serum miR-770, Non-small cell lung cancer, Diagnosis, Prognosis.

Introduction

Lung cancer remains a leading cause of cancer-related mortality and morbidity worldwide, with dismal outcomes and an increasing incidence in China[1,2]. Non-small cell lung cancer (NSCLC) represents 85% of patients diagnosed with lung cancer[3]. Although the survival of NSCLC patients has been significantly improved with current advances in the chemotherapy and molecular targeting therapy, the long-term survival remains poor due to the high rate of recurrence and metastasis[4,5]. Therefore, it is necessary to uncover the key molecules, which help to identify novel diagnostic and prognostic markers to improve patients' survival.

MicroRNAs (miRNAs) are a group of non-coding, single-stranded RNAs that negatively regulate gene expressions at the post-transcriptional level[6]. It has confirmed that miRNAs can bind to the 3' untranslated region of related mRNAs, leading to translational inhibition and/or mRNA degradation[7]. miRNAs have critical roles in regulating important cellular functions such as proliferation, metastasis, apoptosis, and differentiation[8,9]. Several miRNAs have been shown to play important roles in carcinogenesis and tumor metastasis and function either as oncogenes or tumor suppressor genes depending on the type of tumor[10-12]. Up to date, most of researches focused on the biological function and molecular mechanism of miRNAs in tumor tissues. Recently, serum miRNAs have been implicated for potential biomarkers in several diseases, including tumors[13-15]. However, only a few serum miRNAs were investigated in NSCLC patients.

MiR-770 is a novel miRNA and its research is very small. Previous works[16,17] showed that miR-770 expression was down-regulated in several tumors, and its low expression may be associated with cisplatin chemoresistance, suggesting

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that miR-770 may act as a negative regulator in progression of tumors. In addition, down-regulation of miR-770 was also observed in NSCLC tissues[18]. However, the expression pattern and potential clinical significance of serum miR-770 have not been investigated.

Patients and Methods

Patients and Samples Collection

A total of 273 participants, including 196 NSCLC patients and 77 healthy individuals, were recruited for plasma samples from Jining No.1 People's Hospital between January 2008 and March 2012. All patients were diagnosed with NSCLC based on histopathological evaluation. Healthy individuals were within the normal range of all laboratory findings and had no history of cancer. No local or systemic treatment was conducted in these patients previously. Patients characteristics are described in Table I. Overall survival was defined as the time interval between the date of surgery and the date of death. Written informed consent was obtained from all patients, and the research was approved by the Institutional Review Board of First Affiliated Hospital of Jining No.1 People's Hospital.

RNA Extraction and Quantitative Real-Time PCR

Total RNA was isolated from serum using a TRIzol LS isolation kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The reverse transcription reaction was carried out using PrimeScript II cDNA synthesis kit (TaKaRa, Dalian, Niaoning, China). qRT-PCR was performed using UltraSYBR mixture (Cwbio, Haidian, Beijing, China) and conducted using the CFX Connet TM Real-time PCR system (TaKaRa, Dalian, Niaoning, China). GAPDH was used as an internal control and miR-770 values were normalized to GAPDH for relative gene expression quantitation. The fold changes of miR-770 were calculated by the $2^{-\Delta\Delta Ct}$ method. All of the primers were synthesized by Invitrogen (Carlsbad, CA, USA) and sequences were as follows: miR770 forward, 5'-CCAGTACCACGTGTCAG -3' and reverse, 5'-GAACATGTCTGCGTATCTC-3'; GADPH forward, 5'-TCCACCACCCTGTTGCTGTA-3' and 5'-ACCACAGTCCATGCCATCAC-3'.

Statistical Analysis

Statistical analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Mann-Whitney U test was used to compare the difference

Table I. Relationship between the serum miR-770 expression and clinicopathological features of patients with NSCLC.

Characteristics	Case number	Serum miR-770 expression		*p*-value
		High	Low	
Gender				0.169
Male	114	60	54	
Female	82	35	47	
Age (year)				0.458
<60	92	42	50	
≥60	104	53	51	
Tumor size				0.093
T1/T2	116	62	54	
T3/T4	80	33	47	
Site of tumor				0.955
Left lung	78	38	40	
Right lung	118	57	61	
Histological grade				0.015
Moderately	119	66	53	
Poorly	77	29	48	
Tumor stage				0.002
I/II	118	68	50	
III/IV	78	27	51	
Lymph node metastasis				0.007
Negative	128	71	57	
Positive	68	24	44	

of serum miR-770 expression levels. The association between the serum miR-770 and clinicopathologic features was tested using the x^2-test. The diagnostic value of serum miR-770 NSCLC was assessed by the receiver-operating characteristic (ROC) curve analysis. Overall survival curves were plotted using the Kaplan-Meier method and were determined for the statistical significance using a log-rank test. Univariate and multivariate Cox regression analyses were employed to assess survival data. p-value < 0.05 was considered as statistically significant.

Results

Serum miR-770 is Downregulated in NSCLC Patients

To investigate the potential roles of serum miR-770 in NSCLC development, we performed RT-PCR to detect the expression level of serum miR-770 in NSCLC patients and healthy controls. We found that serum miR-770 was downregulated in NSCLC patients compared with healthy controls ($p < 0.001$) (Figure 1). This result indicated that down-regulation of serum miR-770 may contribute to tumorigenesis of NSCLC.

Correlation of Serum miR-770 Expression with Clinicopathological Parameters

To analyze whether serum miR-770 was associated with the progression of NSCLC, we explored its association with clinical characteristics.

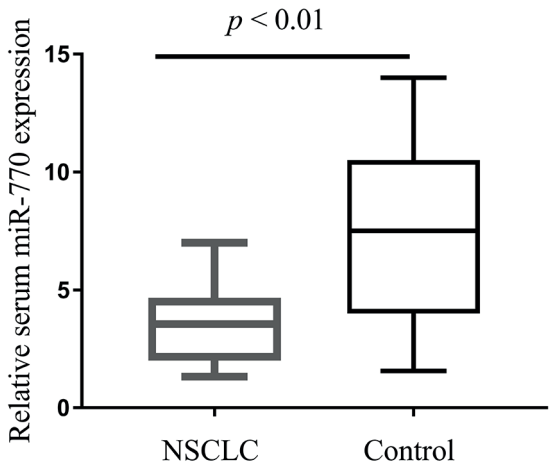

Figure 1. The expression levels of serum miR-770 in NSCLC patients and healthy controls were determined by qRT-PCR. The relative serum miR-770 level in NSCLC patients was significantly lower than that in healthy people ($p < 0.01$).

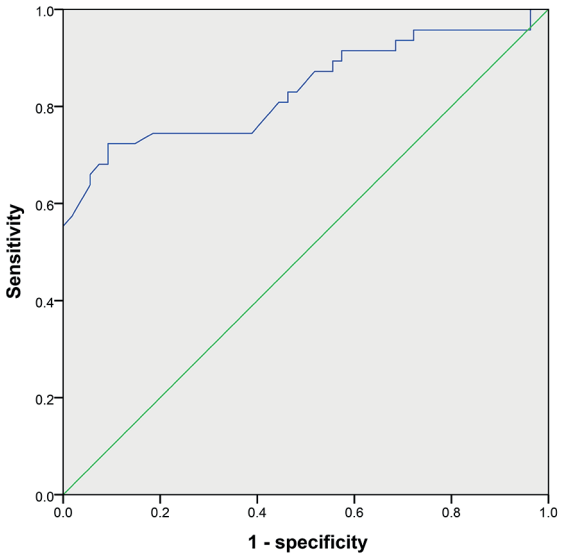

Figure 2. ROC curve analysis for the detection of NSCLC using serum miR-770.

Then, all NSCLC patients were classified into either the low serum miR-770 expression group (N = 95) or the high serum miR-770 expression group (N = 101) according to the median serum miR-770 level. We found that low expression of serum miR-770 was positively associated with histological grade ($p = 0.015$), tumor stage ($p = 0.002$) and lymph node metastasis ($p = 0.007$) in NSCLC patients (Table I). However, no significant differences about other characteristics of patients were found.

Diagnostic Value of Serum miR-770 in NSCLC Patients

In order to explore the potential value of serum miR-770 level as a diagnostic marker for NSCLC, we analyzed the ROC curve. The results indicated that serum miRNA-770 was a potential biomarker for discriminating NSCLC patients from healthy controls, with the AUC value of 0.835 (95 % CI, 0.751-0.919) (Figure 2). The cut-off value of serum miR-770 in NSCLC patients was 5.723. The optimal sensitivity and specificity were 68% and 89%, respectively. Those results suggested that serum miR-770 may be used as a useful biomarker to diagnose NSCLC.

Prognostic Value of Serum miR-770 for NSCLC Patients

We further performed Kaplan-Meier analysis to investigate whether the serum miR-770 expres-

Table II. Univariate and multivariate analysis of prognostic parameters in patients with cervical cancer by Cox regression analysis.

	Univariate analysis		Multivariate analysis	
Parameters	**HR(95%CI)**	***p*-values**	**HR(95%CI)**	***p*-values**
Gender	1.873(0.782-3.261)	0.138	-	-
Age	1.563(0.942-2.893)	0.194	-	-
Tumor size	2.321(0.593-3.884)	0.117	-	-
Site of tumor	1.653(0.705-2.676)	0.679	-	-
Histological grade	3.448(1.213-4.269)	0.019	2.783(0.981-3.263)	0.025
Tumor stage	4.763(1.472-8.372)	0.001	3.676(1.237-6.239)	0.001
Lymph node metastasis	3.259(1.559-5.362)	0.001	2.653(1.273-4.282)	0.005
Serum miR-770 expression	4.337(1.873-8.273)	0.001	3.251(1.573-6.632)	0.003

sion level correlated with the outcome of NSCLC patients. The results showed that NSCLC patients with high serum miR-770 expression had a significantly longer survival time than those with low serum miR-770 expression ($p = 0.000$) (Figure 3). Univariate and multivariate analyses were utilized to further evaluate the prognostic value of serum miR-770 expression. A univariate analysis demonstrated that low serum miR-770 expression (HR 4.337, 95 % CI 1.873-8.273, $p = 0.001$) was associated with poor overall survival of NSCLC patients (Table II). Further assay by multivariate analysis confirmed that serum miR-770 levels (HR 3.251, 95 % CI 1.573-6.632, $p = 0.003$) were independent prognostic factors for overall survival.

Discussion

NSCLC could remain a major health problem for at least the next 30 years. Growing evidence shows that the tumorigenesis and development of NSCLC is a complex process involving various genetic and epigenetic alterations[19,20]. These complicated situations cause it hard to find critical etiology and develop effective therapeutic approach. Clinical practice reveals that the patients diagnosed at an early stage show better outcomes[21]. Up to date, tumor markers, such as carcinoembryonic antigen and serum cytokeratin 19 fragment, have been used for diagnosis of NSCLC[22,23]; however, their accuracy was not satisfying. Thus, there is still an urgent need for the identification of more promising biomarkers. Recently, diagnostic and prognostic value of several serum miRNAs has been reported in various tumors, such as serum miR-195 for osteosarcoma[24], circulating miR-497 for bladder cancer[25] and serum miR-155 for lung cancer[26]. Those results highlighted serum miRNAs as potential biomarkers for early diagnosis of NSCLC.

Accumulating evidence has shown that miR-770 plays a crucial role in tumor biology. For instance, Zhao et al[17] reported that down-regulation of miR-770 may contribute to ovarian cancer progression by promoting cisplatin chemoresistance by modulating ERCC2. Lee et al[27] reported that miR-770 suppressed tumor growth and sensitizes tumors to radiation via negative regulation of PBK. Li et al[28] found that the expression levels of miR-770 was significantly down-regulated in chemo-sensitive tissues and forced expression of miR-770 could inhibit the chemo-resistance and metastasis of breast cancer by targeting STMN1.

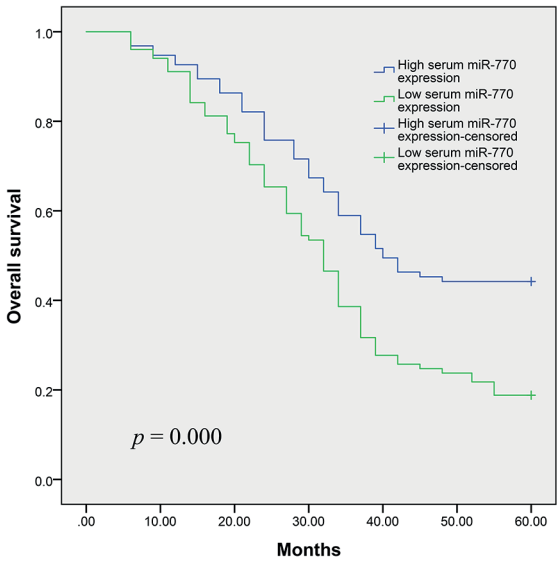

Figure 3. Kaplan-Meier curves for overall survival of 196 NSCLC patients, divided according to serum miR-770 expression levels. Low serum miR-770 expression was significantly associated with poor survival ($p = 0.000$).

In addition, they found that high expression of miR-770 in breast cancer patients was significantly associated with better prognosis. In lung cancer, Zhang et al[18] showed that miR-770 expression was significantly down-regulated in NSCLC tissues compared to normal lung tissues. *In vitro* and *in vivo* assay revealed that miR-770 upregulation could inhibit NSCLC cell proliferation, migration and invasion. Notably, their clinical assay indicated that low expression of miR-770 was significantly associated with poor prognosis of NSCLC patients. Those results suggested miR-770 as a tumor suppressor in progression of NSCLC. However, whether serum miR-770 was dysregulated in NSCLC patients and its prognostic and diagnostic value have not been reported.

In the present study, we firstly detected the expression levels of serum miR-770 in NSCLC patients and healthy controls. The trend of miR-770 in NSCLC tissues as well as the up-regulation of serum miR-770 was also observed in NSCLC patients compared to healthy controls. Then, we explored the relationship between serum miR-770 expression and clinicopathologic features of NSCLC patients, and the results showed that low expression of serum miR-770 was positively associated with histological grade, tumor stage and lymph node metastasis, suggesting that serum miR-770 may be utilized as potential biomarker for predicting prognosis of NSCLC patients. Subsequently, the results of ROC assay revealed that the expression of serum miR-770 could be used to discriminate NSCLC from healthy controls (AUC = 0.835). A five-year follow-up study showed that patients with low serum miR-770 expression showed poorer survival than those with high serum miR-770 expression. More importantly, univariate and multivariate analysis confirmed that low serum miR-770 expression was an unfavorable prognostic factor for NSCLC patients.

Conclusions

We showed for the first time that serum miR-770 expression was significantly associated with NSCLC progression and poor prognosis. Serum miR-770 may represent a novel diagnostic, prognostic biomarker and a potential therapeutic target of NSCLC in the future.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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