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Downregulation of miR-361-5p associates with aggressive clinicopathological features and unfavorable prognosis in non-small cell lung cancer

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Abstract. – OBJECTIVE: To assess the impact of miR-361-5p expression levels on non-small-cell lung cancer (NSCLC) survival.

PATIENTS AND METHODS: A total of 183 patients with NSCLC who underwent surgery between October 2007 and April 2010 were included in this study. Expression levels of miR-361-5p were detected by using qRT-PCR. The association of miR-361-5p expression with clinicopathologic characteristics of NSCLC patients was analyzed. Kaplan-Meier Plotter was performed to identify the prognostic roles of miR-361-5p in NSCLC patients. Finally, multivariate analysis was performed using the Cox proportional hazard analysis.

RESULTS: Results indicated that miR-361-5p was lowly expressed in NSCLC compared with adjacent non-malignant tissues ($p < 0.01$). And low miR-361-5p expression in NSCLC was significantly correlated with TNM stage ($p = 0.000$), lymph node metastasis ($p = 0.001$) and lymphatic invasion ($p = 0.032$). Kaplan-Meier analysis with the log-rank test indicated that low miR-361-5p expression had a significant impact on overall survival ($p < 0.001$). Furthermore, Multivariate analyses indicated that miR-361-5p represented an independent predictor for overall survival of NSCLC ($p = 0.007$).

CONCLUSIONS: Our work revealed that miR-361-5p played critical roles in NSCLC progression and could represent a novel prognostic marker in NSCLC patients.

Key Words:
miR-361-5p, Non-small-cell lung cancer, Prognosis.

Introduction

The incidence of lung cancer remains high, and lung cancer ranks first regarding cancer-related mortality[1]. Patients with non-small cell lung cancer (NSCLC), which accounts for more than 80% of lung cancer, carry a poor clinical outcome with 5-years survival rate of 10%-15%[2,3]. Radical surgery is thought to be the single modality that can provide opportunity for cure and long-time survival[4]. However, long-term survival after surgical resection remains poor owing to the high rate of recurrence and metastasis[5,6]. Exploring novel and special promising predictive factors are still urgent needed to improve the prognosis of NSCLC.

MiRNAs are highly conserved small non-coding regulatory RNAs with sizes of 17-25 nucleotides[7]. In general, miRNAs exert their regulatory role on protein-coding gene expression[8]. Some studies[9,10] have shown that miRNAs contribute to many basic cellular functions including proliferation, differentiation, and death. In human cancer, miRNAs can function as oncogenes or tumor suppressor genes depending on the nature of their targets[11,12]. More and more evidence showed that miRNAs could be useful prognostic marker for human cancer. For instance, Roy et al[13] showed that miR-194 can be used to compliment other biomarkers to predict disease relapse and overall survival. Zhang et al[14] provided evidence that miR-148b expression could be an independent prognostic factor for patients with hepatocellular carcinoma.

MiR-361-5p, which is located on chromosome X, has been reported to play a critical role in several human tumors[15]. In the current study, we examined the expression level of miR-361-5p in NSCLC specimens; next, we analyzed its correlations with clinicopathological characters in order to determine the clinical significance of miR-361-5p in NSCLC. This study may provide a new biomarker and therapeutic

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Table I. Association of miR-361-5p expression levels with clinical factors in NSCLC patients.

Characteristics	All patients	miR-361-5p low expression	miR-361-5p high expression	*p*-value
No.	183	92	91	
Age				0.085
<50 years	73	31	42	
≥50 years	110	61	49	
Gender				0.597
Male	126	65	61	
Female	57	27	30	
Smoking history				0.379
Yes	134	70	64	
No	49	22	27	
TNM stage				0.000
I+II	89	32	57	
III+IV	94	60	34	
Lymph node metastasis				0.001
Yes	80	51	29	
No	103	41	62	
Lymphatic invasion				0.032
Positive	95	55	40	
Negative	88	37	51	
Histological type				0.598
Adenocarcinoma	82	43	39	
Squamous carcinoma	101	49	52	

target that facilitates early diagnosis and treatment of NSCLC.

Patients and Methods

Patients and Tissue Samples

We analyzed tumor specimens from 183 patients with lung cancer who underwent surgery for excision of a primary tumor between October 2007 and April 2010 in the Department of Thoracic Surgery, Yantai Yuhuangding Hospital. The histopathological diagnosis of all samples was respectively diagnosed by two pathologists. TNM staging was based on the seventh edition of the AJCC TNM system. None of the patients had received chemotherapy or radiotherapy before surgery excision. All the patients were Chinese. The clinicopathological information for the patients is summarized in Table I. Written informed consent on the use of clinical specimens from each patient was achieved. This study was approved by the hospital's Ethics Committee.

Quantitative real-time PCR

Total RNA was purified from NSCLC samples and normal control tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Synthesis of cDNA with reverse transcriptase was performed by NCode miRNA quantitative RTPCR Kits (Invitrogen, Carlsbad, CA, USA). The miR-361-5p expression levels were determined by real-time PCR using Taqman primers and Taqman PCR master mixture (Applied Biosystems, Foster City, CA, USA). Expression of miR-361-5p (5'-AAT AGT CTT AGA GGT CCC CATG-3') was normalized to that of the U6 snRNA (5'-GAC CTT AGC AAT AGC ATT GGCA-3'). Each PCR reaction was run in triplicate and gene relative expression was calculated as $2^{-\Delta\Delta Ct}$.

Statistical Analysis

Statistical analysis was performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). The X^2 test was performed to analyze the correlation between miR-361-5p expression and clinicopathological parameters. The log-rank test was used to assess the statistical significance of Kaplan-Meier plots. The Cox proportional hazard model was used to identify whether serum miR-361-5p was an independent risk factor for NSCLC, via a multivariate analysis. $p < 0.05$ was deemed to be statistically significant.

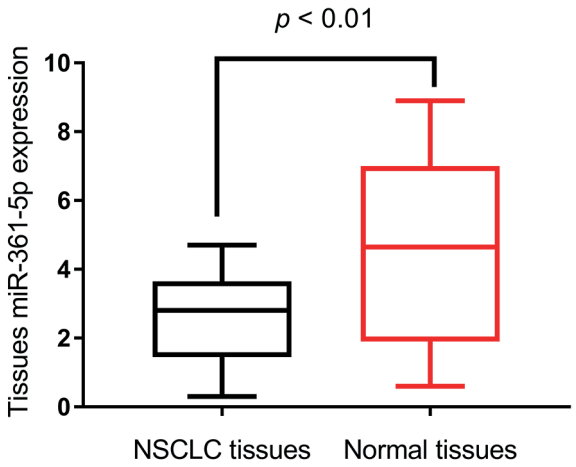

Figure 1. miR-361-5p expression was decreased in NSCLC tissues compared with normal tissues ($p < 0.01$).

Results

Expression of miR-361-5p is Increased in NSCLC tissues.

We first analyzed the expression of miR-361-5p in 183 human NSCLC tissues with paired adjacent normal tissues by RT-PCR. As shown in Figure 1, the expression of miR-361-5p was significantly downregulated in NSCLC tissues compared with adjacent non-cancerous tissues ($p < 0.01$)

Correlations Between Patients' Characteristics and miR-361-5p Expression

The median value of miR-361-5p level in 183 osteosarcoma patients (4.16) was used as the cutoff point to divide these patients into miR-361-5p-low (n = 92) and miR-361-5p-high (n = 91) groups. The association of miR-361-5p with clinicopathological characteristics was analyzed in NSCLC patients via X^2-test. As shown in Table I, low expression of miR-361-5p was significantly associated with TNM stage ($p = 0.000$), lymph node metastasis ($p = 0.001$) and lymphatic invasion ($p = 0.032$). However, statistical analysis showed no significant correlation between miR-361-5p expression and age, gender, smoking history or histological type.

Table II. Multivariate analysis of prognostic factors in for overall survival.

Variables	Hazard ratio	95% CI	*p*-value
Age	1.352	0.463-3.239	0.373
Gender	0.915	0.317-2.831	0.528
Smoking history	2.831	0.935-3.655	0.317
TNM stage	3.893	1.936-8.933	0.014
Lymph node metastasis	3.347	2.316-7.744	0.008
Lymphatic invasion	2.783	1.045-4.159	0.155
Histological type	1.673	0.944-2.815	0.349
miR-361-5p	3.318	1.894-8.216	0.007

The Association Between the Expression Level of miR-361-5p and Prognosis.

To elucidate the prognostic value of miR-361-5p expression, Kaplan-Meier survival analysis was applied to compare overall survival according to the miR-361-5p expression. The results demonstrated that high expression of miR-361-5p in tumor tissues showed a survival benefit in NSCLC patients (Figure 2). To further investigate whether the expression of miR-361-5p is an independent prognostic factor for glioma. In a multivariate Cox regression analysis, we found that low miR-361-5p expression was significantly associated with shorter overall survival of NSCLC patents (HR=3.318; $p = 0.007$, Table II). These results showed that the expression of miR-361-5p was an independent prognostic factor for NSCLC.

Discussion

A lot of studies indicated that exploring potential biomarker for the diagnosis and prognosis of the tumor could be beneficial for the development of treatments for tumor. The sensitivities and spe-

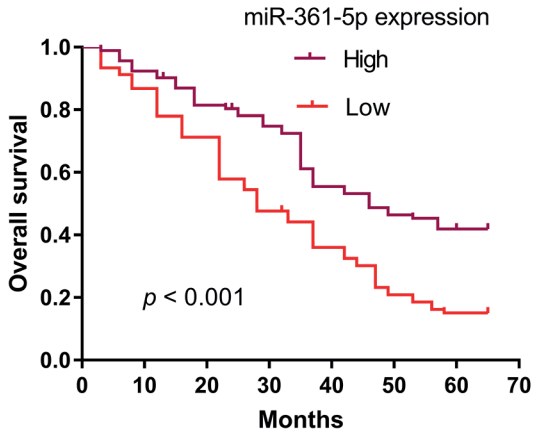

Figure 2. Kaplan-Meier analysis for survival based on miR-361-5p expression.

cificities of current clinicopathological parameters such as Lymph node metastasis, tumor grade, and tumor size are not satisfactory. miRNAs have been hypothesized[16] as potential biomarkers for cancer prognosis due to their aberrant expression profile in cancerous tissue. Here, we focus on miR-361-5p and our aim is to investigate the clinical values of this miR-361-5p in NSCLC.

Up to date, the effect of miR-361-5p had been reported in several different tumors. For instance, Sun et al[17] found that miR-361-5p was down-regulated in hepatocellular carcinoma tissues in comparison to adjacent normal tissues. Forced expression of miR-361-5p significantly inhibited tumor growth in the nude mice. Ma et al[18] reported that ectopic expression of miR-361-5p suppressed tumor cell proliferation and metastasis-related traits *in vitro* as well as in *vivo*, supporting an anti-cancer role of this miRNA in colorectal and gastric cancer progression. In the study by Yang et al[19], miR-361-5p suppressed cell invasion and migration by targeting by targeting signal transducer and activator of transcription-6 (STAT6) in human pancreatic cancer. Recently, Ma et al[20] found that the expression of miR-361-5p was significantly lower in NSCLC tissues compared with that in adjacent tissues. Furthermore, they showed that upregulation of miR-361-5p depressed proliferation and colony formation via the STAT6/Bcl-xL pathway *in vitro* and in *vivo*. Together, these previous researches have demonstrated the important role of miR-361-5p in various cancers.

In the present paper, we found that miR-361-5p expression was significantly decreased in NSCLC tissues compared with adjacent tissues and it was significantly associated with pathological stage. To identify the prognostic value of miR-361-5p expression in NSCLC patients, we performed Kaplan-Meier analysis. Interestingly, we found similar results with Cao et al[21]. The results showed that the overall survival rates were significantly lower in low miR-361-5p expression group than those in the high miR-361-5p expression group. According to multivariate analyses miR-361-5p was an independent unfavorable prognostic biomarker for NSCLC patients.

Conclusions

Our findings showed that detection of miR-361-5p level might serve as a clinical predictor in the prediction of clinical outcomes for the patients with NSCLC. Further studies are needed to investigate the mechanism underlying the inhibitory function of miR-361-5p.

Conflict of Interest

The authors declare no conflicts of interest.

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