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Increasing expression of miR-5100 in non-small-cell lung cancer and correlation with prognosis

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Abstract. – OBJECTIVE: A previous study indicated that miR-5100 served as a tumor oncogene in lung cancer. However, whether miR-5100 may serve as a novel prognostic marker in non-small cell lung cancer (NSCLC), has not been investigated. The aim of this study was to investigate miR-5100 expression and its clinical significance in NSCLC patients.

PATIENTS AND METHODS: Expression of miR-5100 was detected in NSCLC tissues and matched normal lung tissues by quantitative Real-time polymerase chain reaction. The correlation between miR-5100 expression and clinical features were statistically analyzed. Survival rate was analyzed by log-rank test, and survival curves were plotted according to Kaplan-Meier. The correlation between miR-5100 expression and prognosis of NSCLC patients was further evaluated by univariate and multivariate analysis.

RESULTS: As revealed by qRT-PCR analysis, the relative level of miR-5100 expression in NSCLC tissues was significantly upregulated, compared with that in corresponding noncancerous tissues ($p < 0.01$). Additionally, high miR-5100 expression was statistically associated with higher clinical stage ($p < 0.001$), N classification ($p = 0.003$) and M classification ($p = 0.027$), but lower differentiated degree ($p < 0.001$). Furthermore, the results of Kaplan-Meier suggested that NSCLC patients with higher miR-5100 expression had significantly poorer overall survival ($p < 0.0001$) and progression-free survival ($p < 0.0001$). Multivariate survival analysis verified that miR-5100 expression level was an independent predictor of both overall survival and progression-free survival for NSCLC patients.

CONCLUSIONS: Our data suggested that up-regulation of miR-5100 was correlated with NSCLC progression, which provided a potential prognostic biomarker and therapeutic target.

Key Words:
miR-5100, NSCLC, Prognosis, Overall survival, Progression-free survival.

Introduction

Lung cancer is one of the most commonly diagnosed cancers in both women and men, and it is a major cause of malignant tumor-related death worldwide[1]. It is reported that there are more than 1.8 million new cases and almost 1.6 million deaths estimated in 2012[2]. Non-small-cell lung cancer (NSCLC) accounts for approximately 80-85% of lung cancers[3]. Despite newly-developed multi-agent chemotherapy and gradually improved surgical techniques, the five year survival rate of NSCLC patients remains poor[4,5]. More importantly, the prognosis for patients with advanced NSCLC, which does not respond to conventional chemotherapies, remains very poor[6]. Exploring novel and sufficiently effective predictive markers is still urgent needed to improve the prognosis of NSCLC. MicroRNAs (miRNAs) are small non-coding RNAs of 20-22 nucleotides involved in the regulation of gene expression at a post-transcriptional level[7]. Previous evidence[8,9] have showed that miRNAs are involved in various biological processes including cell proliferation, apoptosis and differentiation. In recent years, many miRNAs were observed to participate in the carcinogenesis by modulating important tumor-associated genes[10]. For instance, Chen et al[11] reported that upregulation of miR-664 enhanced, whereas downregulation of miR-664 inhibited the proliferation of osteosarcoma cells via downregulation of FOXO4. Yan et al[12] found that miR-155 promoted the progression of glioma by enhancing the activation of Wnt pathway. Liu et al[13] indicated that upregulation of miR-137 inhibited the proliferation of NSCLC cells by targeting TGFA. Previously Wang et al[14] observed that miR-5100 was overexpressed in a subset of lung cancer tissues. However, the potential role of miR-5100

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in develops and progression of NSCLC remains largely unknown. In the present study, we aimed to explore the prognostic value of miR-5100 in NSCLC patients.

Patients and Methods

Patients and Tissue Samples

Two hundred and thirty patient-derived paired NSCLC and adjacent non-tumorous tissue samples were collected at the Chinese PLA General Hospital. All the tissues were frozen by liquid nitrogen while the serum samples were put into blood collection tube of EDTA and stored at -80°C for RNA extraction. None of the patients had received any therapeutic procedures prior to this study. The histological diagnosis was evaluated by two independent pathologists according to the WHO classification. Overall survival (OS) was defined as the time interval between the date of diagnosis and the date of death. Progression-free survival (PFS) was defined as the time interval between the date of diagnosis and the date of disease relapse. This study was approved by the Ethics Committee of Chinese PLA General Hospital and written informed consent was obtained from all the participants.

Quantitative Real-time PCR

Total RNA was isolated using Invitrogen TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's protocol. cDNA was synthesized using the TaqMan miRNA reverse transcription kit (TaKaRa, Otsu, Shiga, Japan), and expression levels of miR-5100 were quantified using Real-time PCR. Real-time quantitative RT-PCR was performed using the miScript Reverse Transcription and miScript SYBR Green PCR Kit, according to the manufacturer's protocol (Foster City, CA, USA). The mixtures were incubated at 95°C for 10 min, 95°C for 15 s, and 60°C for 1 min (45 cycles). The cycle threshold (CT) values were calculated with SDS 2.4 software (Applied Biosystems, Foster City, CA, USA). Primers used in the experiments were purchased from Invitrogen (Waltham, MA, USA). GDPAH was used for normalization. All reactions were done in triplicate. The RNA expression data were analyzed using comparative Ct (2-ΔΔCt) methods.

Statistical Analysis

All statistical analyses were carried out using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). The significance of miR-5100 in cancers and their corresponding non-tumor colon tissues was assessed by the paired Wilcoxon test. The relationships between clinical parameters and the expression of miR-5100 were calculated using χ^2 tests. The survival rates after tumor removal were calculated by the Kaplan-Meier method. The Cox proportional hazards model was used to calculate the hazard ratios (HRs) and their 95% confidence intervals (95% CIs) of covariates in the analyses of progression-free survival (PFS) or overall survival (OS). $p \leq 0.05$ was accepted as indicating a significant difference in mean values.

Results

MiR-5100 is Overexpressed in NSCLC

To explore the possible role of miR-5100 in NSCLC development, we detected the expression levels of miR-5100 in NSCLC tissues and matched normal tissues. As shown in Figure 1, miR-5100 levels were significantly higher in NSCLC tissues compared with matched non-cancerous tissues ($p < 0.01$), suggesting that miR-5100 is involved in lung oncogenesis.

Correlation Between miR-5100 Expression and Clinical Features and Prognosis of NSCLC Patients

To investigate the association between miR-5100 expression and clinicopathological parameters, the 230 NSCLC tissue samples were divi-

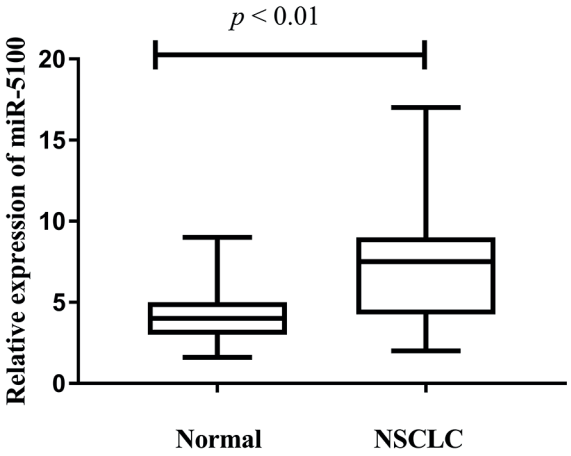

Figure 1. Relative expression of miR-5100 in NSCLC tissues compared with adjacent non-tumor normal tissues. MiR-5100 expression was examined by qRT-PCR and normalized to GAPDH expression.

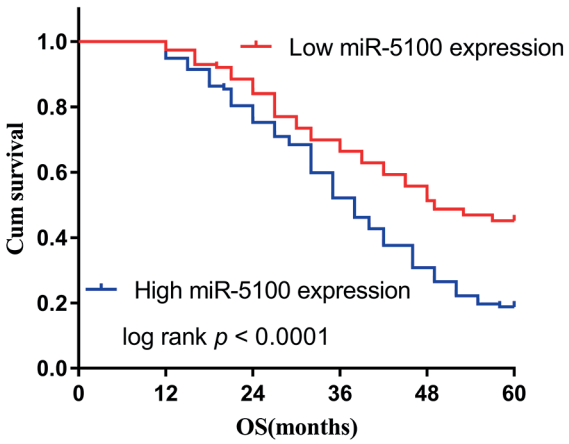

Figure 2. Kaplan-Meier curves for overall survival in patients with NSCLC divided according to miR-5100 expression levels.

ded into two subgroups based on their miR-5100 expression (high and low miR-5100 expression). As summarized in Table I, there were no significant associations between miR-5100 expression and gender ($p = 0.307$), age (y) ($p = 0.425$), smoking ($p = 0.558$) or T classification ($p = 0.066$). However, high miR-5100 expression was statistically associated with higher clinical stage ($p < 0.001$), N classification ($p = 0.003$) and M classification ($p = 0.027$), but lower differentiated degree ($p < 0.001$).

High miR-5100 Expression is Associated with Poor Prognosis in Patients with NSCLC

To explore the prognostic significance of high miR-5100 levels, the survival of patients with high miR-5100 expression was compared to that of patients with low expression. In the Kaplan-Meier survival analysis, NSCLC patients with higher miR-5100 expression had significantly poorer overall survival ($p < 0.0001$, Figure 2) and progression-free survival ($p < 0.0001$, Figure 3). The univariate analyses showed statistically significant associations between OS and differentiated degree (HR=4.731; $p < 0.001$), clinical stage (HR=3.822; $p = 0.001$), N classification (HR=3.631; $p = 0.004$), M classification (HR=0.009; $p = 0.009$) and miR-5100 expression (HR=3.671; $p = 0.001$, Table II). Furthermore, multivariate analyses confirmed that miR-5100 expression was independent prognostic

Table I. Correlations of clinicopathological parameters and expression level of miR-5100 in patients with NSCLC (n=230).

Characteristics	n	miR-5100 (%)		p
		Low expression	High expression	
Gender				0.307
Female	88	47	41	
Male	142	66	76	
Age (y)				0.425
<50	112	52	60	
≥50	118	61	57	
Smoking				0.558
No	94	44	50	
Yes	136	69	67	
Differentiated degree				<0.001
High or Middle	143	84	59	
Low	87	29	58	
Clinical stage				<0.001
I-II	162	92	70	
III-IV	68	21	47	
T classification				0.066
T1-T2	112	62	50	
T3-T4	118	51	67	
N classification				0.003
N0-N1	149	84	65	
N2-N3	81	29	52	
M classification				0.027
M0	177	94	83	
M1	53	19	34	

Table II. Univariate and multivariate analyses for overall survival in NSCLC patients.

Variable	Univariate			Multivariate		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Gender	0.813	0.488-1.549	0.411	-	-	-
Age (y)	1.213	0.783-1.775	0.299	-	-	-
Smoking	1.374	0.557-1.531	0.355	-	-	-
Differentiated degree	4.731	1.781-6.883	<0.001	3.893	1.474-5.842	<0.001
Clinical stage	3.822	1.931-5.572	0.001	3.113	1.462-4.673	0.003
T classification	1.731	0.922-2.231	0.081	-	-	-
N classification	3.631	1.554-4.982	0.004	2.893	1.236-4.116	0.007
M classification	3.131	1.613-4.997	0.009	2.131	1.226-4.131	0.016
miR-5100 expression	3.671	1.774-5.893	0.001	3.113	1.492-4.893	0.003

factors for patients with NSCLC (HR=3.113; 95% CI 1.492-4.893, p = 0.003). In addition, similar findings were observed in PFS (Table III). Taken together, miR-5100 expression were independent prognostic factors for the predicting OS and PFS of NSCLC patients.

Discussion

NSCLC accounts for the greatest proportion of cancer deaths worldwide and its incidence continues to rise in China[15]. Because of its nonspecific symptoms, the diagnosis of NSCLC is usually made postoperatively on tumors at an advanced stage, this is the main reason causing the poor prognosis of NSCLC patients[16,17]. Although several clinicopathological features have been the standard for determining the clinical outcome of NSCLC patients, these methods showed the relatively low specificity[18]. Therefore, it is required to identify effective biomarkers, and therapeutic targets for NSCLC. Extensive studies identified many markers which may be associated with prognosis of NSCLC patients[19,20]. However, up to now, most of these markers had not been proven to be sufficiently effective[21]. Recently, miRNAs became a research hot because its critical role and steady expression in progression of NSCLC. In the present study, we focused on miR-5100. Previous researches demonstrated that miRNAs was deregulated in types of cancers and could be potential prognostic biomarkers for NSCLC. For instance, Shen et al[22] reported that miR-145 was significantly down-regulated in NSCLC tissues compared to normal adjacent laryngeal tissues, and its downregulation was significantly correlated with patient five-year survival. Shang

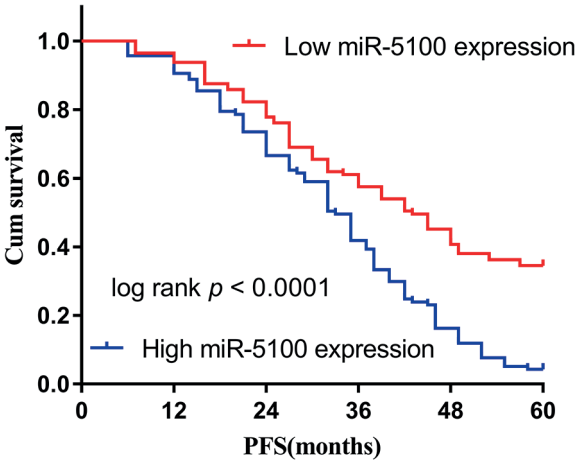

Figure 3. Kaplan-Meier curves for progression-free survival in patients with NSCLC divided according to miR-5100 expression levels.

et al[23] found that low levels of miR-383 was significantly associated with NSCLC patients' poor prognosis, including advanced TNM stages, positive lymph node metastasis, and shorter OS. These findings revealed the potential of miRNAs as a useful marker for prognosis of NSCLC patients. Recently, Chijiiwa et al[24] observed that the expression levels of miR-5100 was up-regulated in pancreatic cancer cells, and overexpression of miR-5100 suppressed pancreatic cancer cell proliferation, migration and invasion by targeting PODXL. However, Huang et al[25] revealed that miR-5100 was highly expressed in lung cancer tissues and lung cancer cell lines. Further *in vitro* and *in vivo* experiments showed that forced expression of miR-5100 promoted cell proliferation and colony formation of lung cancer, whereas inhibition of miR-5100 had the opposite effect. Those different results indicated that miR-5100 served as

Table III. Univariate and multivariate analyses for PFS in NSCLC patients.

Variable	Univariate			Multivariate		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Gender	0.745	0.413-1.631	0.389	-	-	-
Age (y)	1.126	0.664-1.581	0.217	-	-	-
Smoking	1.451	0.667-1.721	0.415	-	-	-
Differentiated degree	4.113	1.561-6.041	<0.001	3.893	1.474-5.842	<0.001
Clinical stage	3.131	1.655-4.836	0.001	2.744	1.231-4.231	0.006
T classification	1.413	0.821-2.011	0.093	-	-	-
N classification	3.131	1.211-4.561	0.002	2.413	1.421-3.893	0.004
M classification	2.761	1.341-4.521	0.006	1.893	1.412-3.782	0.009
miR-5100 expression	3.541	1.521-5.113	0.001	2.893	1.221-4.421	0.002

oncomiRNA or tumor suppressor depending on cancer type. In the present work, we aimed to investigate the clinical significance of miR-5100 in NSCLC. Constant with previous study, our results also showed that the relative level of miR-5100 expression in NSCLC tissues was significantly upregulated, compared with that in corresponding noncancerous tissues. Then, potential associations of miR-5100 expression with clinicopathological factors were examined. We found that high miR-5100 expression was statistically associated with higher clinical stage, N classification and M classification, but lower differentiated degree. In addition, Kaplan–Meier analyses show that NSCLC tissues with high miR-5100 expression levels had poorer OS and PFS. Furthermore, Cox regression analysis proved that miR-5100 could be an independent prognostic indicator for NSCLC patients. Taken together, these findings demonstrated for the first time the clinical significance of miR-5100 in NSCLC.

Conclusions

Our data provided the convinced evidence that overexpression of miR-5100 was correlated with advanced tumor progression and poor clinical prognosis of patients with NSCLC. More in-depth and larger scale studies are required to confirm the correlation between miR-5100 and NSCLC

Conflict of interest

The authors declare no conflicts of interest.

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