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Down-regulation of miR-5702 is associated with clinical progression and poor prognosis in patients with non-small-cell lung cancer

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Abstract. – OBJECTIVE: Significant down-regulation of miR-5702 and its tumor-suppressive roles in non-small-cell lung cancer (NSCLC) have been reported previously. However, its clinical significance in NSCLC has not yet been reported. In this study, we aimed to identify the prognostic value of miR-5702 in NSCLC patients.

PATIENTS AND METHODS: The expression levels of miR-5702 were detected by Real-time quantitative polymerase chain reaction (RT-qPCR). The chi-square test was used for the examination of relationship between miR-5702 expression and clinicopathologic factors. The association between miR-5702 expression and overall survival in patients with NSCLC was statistically analyzed by Kaplan-Meier. Finally, univariate and multivariate models were conducted to determine the prognostic value of miR-5702 in NSCLC patients.

RESULTS: MiR-5702 expression was lowly expressed in NSCLC tissue specimens compared with adjacent nontumor tissue ($p < 0.01$). It was observed that low expression of miR-5702 was associated with clinical stages ($p = 0.029$), lymph node metastasis ($p = 0.016$) and distant metastasis ($p = 0.004$). Moreover, Kaplan-Meier assay showed that patients with lower miR-5702 expression had worse overall survival time than that with higher miR-5702 expression ($p = 0.0097$). Finally, multivariate analysis showed that low expression of miR-5702 was independently associated with overall survival of NSCLC patients (HR = 3.128; 95 % CI: 1.237-5.668, $p = 0.005$).

CONCLUSIONS: Our data, for the first time, indicated that miR-5702 expression may be considered as a prognostic biomarker in NSCLC patients.

Key Words
miR-5702, Non-small-cell lung cancer, Prognosis.

Introduction

Lung cancer, occurring in epithelia of tunica mucosa bronchiorum, is the most common type of cancer and the primary cause of cancer-related death globally in both men and women[1]. Non-small cell lung cancer (NSCLC), the most frequent type of lung cancer, accounts for approximately 80-85% of all cases[2]. Surgical resection is the most suitable therapeutic tool for NSCLC patients diagnosed at an early stage; however, not all lung cancers are suitable for surgery[3]. Because of the absence of specific symptoms and the lack of early detection, many NSCLC patients have gotten a later stage when they came to hospital for cures[4,5]. Although therapy approaches such as radiotherapy, chemotherapy and targeted therapy have been improved in recent years, the prognoses of NSCLC patients remain to be poor (around 10% at 5 years)[6,7]. Tumor recurrence and metastasis are great challenges in the clinical treatment of NSCLC[8,9]. Therefore, there is an urgent need to identify new biomarkers, which may provide new strategies for the diagnosis, prognosis and treatment of NSCLC patients. MicroRNAs (miRNAs) are recently identified noncoding small RNAs of 19-25 nucleotides which are posttranscriptional regulators of gene expression by binding to the 3'-untranslated region (3'-UTR) of their target genes mRNAs[10]. It has been well documented via increasing experiments evidences that miRNAs are positively involved in cellular progression, such as proliferation, cell cycle, apoptosis, differentiation and metabolism[10,11]. Recently, growing evidence from clinical and basic studies had indicated that miRNAs play important roles in tumor tumorigenesis and progression of tumors, and can function as oncogenes or tumor suppressors according to the

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specific roles in tumors[12-14]. In addition, more and more miRNA profiling analysis of NSCLC showed many abnormally expressed miRNAs that are commonly found in NSCLC tissues[15,16]. Therefore, to fully understand the complex mechanisms of NSCLC progression and explore the potential biomarker for diagnosis and prognosis, the function of miRNAs must be considered. miR-5702 is a new miRNA identified to be anti-oncogenic in NSCLC[17]. Previous results of miRNA profiling analysis indicated that miR-5702 was dysregulated in several tumors, such as glioblastoma[18] and metastatic prostate cancer[19]. However, to our best knowledge, the clinical significance of miR-5702 has not been reported. In the current study, we further detected whether miR-5702 was dysregulated in NSCLC and firstly performed statistical analysis to explore the prognostic value of miR-5702 in NSCLC patients. Our findings provided important evidence that miR-5702, together with other biomarkers, could be used to predict the prognosis of NSCLC patients.

Patients and Methods

Patients and Clinical Specimens

A total of 162 patients diagnosed as NSCLC were selected from Jining No.1 People's Hospital during March 2011 to April 2013. All tissue samples were fixed in 10% formalin and embedded in paraffin. None of them had received any adjuvant chemotherapy or radiotherapy. Pathologists confirmed the diagnoses of these NSCLC samples. The overall survival was recorded from the date of diagnosis to the time of the last follow-up or cancer-related death. The clinicopathological factors of all patients are presented in Table I. This study was performed with the approval of the Research Ethics Committee of Jining No.1 People's Hospital. All NSCLC tissue samples included in this investigation were obtained with patients' written informed consent.

Quantitative Real Time-PCR (qRT-PCR) Analysis

The TRIzol reagent (Invitrogen, Carlsbad, CA, USA) was used to extract the total RNA from the NSCLC tissues and matched normal lung tissues. cDNA was synthesized using the PrimeScript RT Reagent Kit (TaKaRa, Otsu, Shiga, Japan). RT-PCR was performed with the SYBR Premix EX Taq (TaKaRa, Otsu, Shiga, Japan) using an ABI 7500 Real-Time PCR system (Biosystems, Foster City, CA, USA). Real-time PCR was performed under the following conditions: 95°C, 10 min; 95°C, 15 s; 60°C, 1 min (40 cycles). GAPDH (glyceraldehyde 3-phosphate dehydrogenase, RiboBio) were used as an internal control. Fold changes were calculated through the relative quantification $2^{-\Delta\Delta Cq}$ method. The primers were as follows: miR-5702 forward, 5'-GCTGAGTCAGCAACATAT-3'; reverse, 5'-ATGACCCCAAAAGCGGGACT-3'. GAPDH forward, 5'-TGAAGGTCGGAGTCAACGGA-3'; reverse: 5'-CCTGGAAGATGGTGATGGGAT-3'.

Statistical Analysis

Statistical analysis was performed using SPSS software 17.0 (SPSS Inc., Chicago, IL, USA). The difference between the 2 groups was analyzed by Students' *t*-test. The relationship between miR-5702 expression and clinicopathologic characteristics was analyzed using the chi-square test. Survival analyses were performed using the Kaplan-Meier and the log-rank tests. The significance of survival variables was analyzed using the Cox multivariate proportional hazards model. A p-value < 0.05 was considered statistically significant.

Results

miR-5702 is Downregulated in NSCLC Patients

Previously, down-regulation of miR-5702 has been reported in NSCLC tissues and cell lines. However, the data was limited and further experiments were needed to confirm previous results. Then, we collected NSCLC tissues and matched normal lung tissues from 162 NSCLC patients in our hospital, and RT-PCR was performed. As shown in Figure 1, our statistics results showed

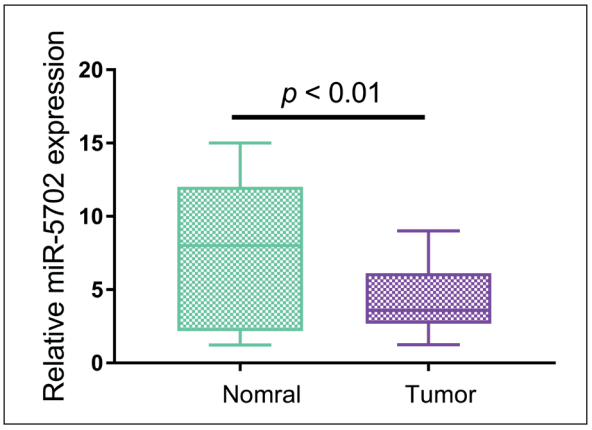

Figure 1. The expression level of miR-5702 in NSCLC (n = 162) or adjacent normal lung tissues (n = 162) was determined by RT-qPCR analysis.

that miR-5702 expression in NSCLC tissues was significantly lower than that in matched adjacent nontumor lung tissues ($p < 0.01$). Thus, once again we also reported that miR-5702 expression was down-regulated in NSCLC patients.

Correlations Between miR-5702 and Clinical Features of NSCLC Patients

To explore the association between miR-5702 expression and clinicopathological parameters, all 162 NSCLC tissue samples were divided into two subgroups (High group and Low group) based on their miR-5702 expression. As shown in Table I, our data by performing chi-square analysis indicated that low miR-5702 expression levels were positively associated with clinical stages ($p = 0.029$), lymph node metastasis ($p = 0.016$) and distant metastasis ($p = 0.004$) of NSCLC. However, no statistically significant correlation was observed between miR-5702 expression and other clinicopathological factors. Our results highlighted the suppressive roles of miR-5702 in clinical progression of NSCLC patients.

Low-Expression Level of miR-5702 Predicts Poor Prognosis in NSCLC Patients

Then, we further performed Kaplan-Meier analysis to explore the prognostic value of miR-5702 in NSCLC patients. As shown in Figure 2, our data showed longer survival times for patients with high miR-5702 expression by comparing with patient of low miR-5702 expression ($p = 0.0097$). Moreover, univariate and multivariate analyses were used to analyze the impact of miR-5702 expression and other clinicopathological features on prognosis of NSCLC patients. It was observed via univariate analyses that low miR-5702 expression ($p = 0.001$), clinical stages ($p = 0.014$), lymph node metastasis ($p = 0.003$) and distant metastasis ($p = 0.001$) were significantly correlated with overall survival of NSCLC patients (Table II). More importantly, the results of multivariate analyses confirmed miR-5702 expression (RR=3.128, 95% CI: 1.237-5.668, $p = 0.005$) as an independent prognostic biomarker for NSCLC patients.

Table I. Analysis of the variables between the group of healed and failures patients.

Variable	No.	miR-5702 expression		p
		High	Low	
Age (years)				0.641
< 60	84	40	44	
≥ 60	78	40	38	
Gender				0.542
Male	105	50	55	
Female	57	30	27	
Tumor size (cm)				0.195
< 3	93	50	43	
≥ 3	69	30	39	
Histology				0.630
Adeno	76	36	40	
Squamous	86	44	42	
Smoking history				0.590
Smokers	104	53	51	
Never smoke	58	27	31	
Clinical stages				0.029
I-II	99	56	43	
III-IV	63	24	39	
Lymph node metastasis				0.016
No	109	61	48	
Yes	53	19	34	
Distant metastasis				0.004
No	108	62	46	
Yes	54	18	36	

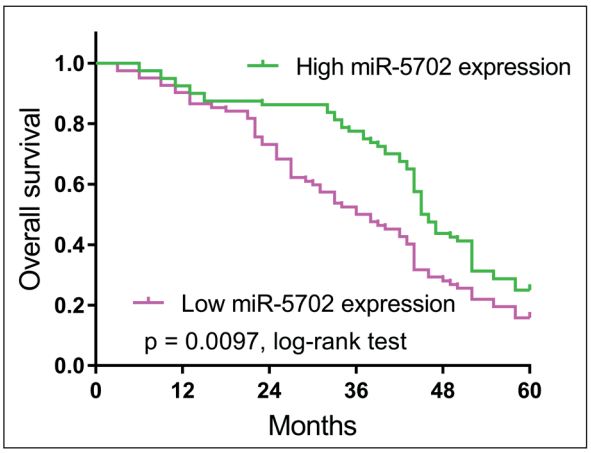

Figure 2. Overall survival curves for two groups defined as low and high expression of miR-5702 in NSCLC patients. Lower miR-5702 expression was significantly associated with unfavorable prognosis of NSCLC patients ($p < 0.0097$, log-rank test).

Discussion

NSCLC has become the number one killer among cancers worldwide and its incidence is decreasing in China[20]. The metastatic cascade is a complex, highly inefficient, but deadly process and NSCLC frequently spreads to bone, with metastases evident at post-mortem in up to 36% of patients[21,22]. Identification of biomarkers related to metastasis was very critical for the treatment of NSCLC patients because individualized treatments rely on the predication of prognosis of patients[23]. Some of these biomarkers have been subjected to extensive independent validation. However, only a few biomarkers have been used in clinical use due to a relatively low diagnostic and prognostic accuracy[24-26]. Recently, the possibility of miRNAs as potential biomarkers attracted growing attention due to its frequent dysregulation and important biological functions in tumor[27,28]. In this study, our attention focused on a newly miRNA termed as miR-5702. Previously, increasing studies had reported that miRNAs can act as oncogenes or tumor suppressors in NSCLC depending on their target genes. For instance, Liang et al[29] reported that miR-18a-5p expression was up-regulated in NSCLC and its forced expression could suppress NSCLC cell proliferation and invasion by directly targeting IRF2 which was an important regulator in progression of NSCLC. Mao et al[30] showed that miR-187-5p was also lowly expressed in NSCLC and associated with advanced TNM stage and poor prognosis of NSCLC patients. *In vitro* and *in vivo* indicated that overexpression of miR-187-5p inhibited the growth and invasion of NSCLC cells by down-regulation of CYP1B1. On the other hand, Sun et al[31] revealed that miR-346 was up-regulated in NSCLC and associated with shorter overall survival, furthermore, they suggested that knockdown of miR-346 promoted cell growth and metastasis by regulation of XPC/ERK/Snail pathway. Recent study by Zhang et al[17] firstly reported that the expression levels of miR-5702 was significantly down-regulated in NSCLC. Moreover, they showed that up-regulation of

Table II. Prognostic factors in Cox proportional hazards model.

Variable	Univariate analysis			Multivariate analysis		
	RR	95% CI	*p*	RR	95% CI	*p*
Age ≥60 *vs.* <60	0.863	0.523-1.335	0.367	–	–	–
Gender Male vs. Female	1.321	0.746-1.973	0.213	–	–	–
Tumor size <3 vs. ≥3	1.367	0.815-2.138	0.144	–	–	–
Histology Adeno *vs.* Squamous	1.546	0.472-1.995	0.167			
Smoking history Smokers vs. Never smoke	1.213	0.589-1.994	0.139	–	–	–
Clinical stages I–II vs. III–IV	2.895	1.452-4.885	0.014	2.427	1.233-4.123	0.027
Lymph node metastasis No vs. Yes	3.127	1.472-5.213	0.009	2.654	1.168-4.437	0.017
Distant metastasis No vs. Yes	3.653	1.512-6.442	0.003	3.216	1.237-5.467	0.008
miR-5702 expression High *vs.* Low	3.677	1.478-6.445	0.001	3.128	1.237-5.668	0.005

miR-5702 suppressed NSCLC cells proliferation and invasion by targeting ZEB1, suggesting miR-5702 as a tumor suppressor in NSCLC. However, whether miR-5702 acted as regulator to influence the prognosis of NSCLC patients has not been investigated. In this study, we sought to identify whether miR-5702 was down-regulated in a large sample size of NSCLC tissues, finding that significant down-regulation of miR-5702 in NSCLC tissues compared to matched normal lung tissues, which was consistent with previous results by Zhang et al[17]. Next, for the first time, we analyzed the association between miR-5702 expression and clinicopathologic features in NSCLC patients by performing chi-square test, finding that lower expression of miR-5702 was significantly associated with advanced clinical stages, lymph node metastasis and positively distant metastasis, suggesting that miR-5702 may be used as potential biomarkers. Furthermore, the results of Kaplan-Meier assay suggested that patients with lower miR-5702 expression showed lower overall survival rate, revealing that miR-5702 levels may be related to the long-term overall. Finally, we performed univariate and multivariate analysis to explore the clinical value of miR-5702 as an independent prognostic factor for NSCLC patients, and the results confirmed our hypothesis. However, because of limited samples, the larger simple sizes were needed to confirm our findings. On the other hand, the potential mechanism by which miR-5702 exhibited its tumor-suppressive roles remains to be further elucidated.

Conclusions

We revealed for the first time that miR-5702 is downregulated in NSCLC patients and associated with unfavorable prognosis, thereby potentially representing a novel prognostic biomarker for NSCLC patients.

Conflict of Interests

The Authors declare that there are no conflicts of interest.

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