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Original Article

Down-regulation of miR-182 and miR-183 acts as potential predictor biomarkers in progression, metastasis, and poor prognosis of non-small cell lung cancer

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Abstract: microRNAs (miRNAs) play important roles in cancer development, progression, and metastasis. The aim of this study was to explore the expression of miR-182 and miR-183 and its potential relevance to clinicopathological features and patient survival. miRNAs expression in 73 pairs of NSCLC tissues and their adjacent non-tumor tissues was measured by quantitative real-time PCR (qRT-PCR). Additionally, the correlation of miR-182 and miR-183 expression with clinicopathological features and prognosis of NSCLC patients were analyzed. Our results suggested that miR-182 expression was significantly down-regulated in NSCLC tissues compared with adjacent non-tumor tissues. On the other hand, miR-183 expression was also clearly down-regulated in NSCLC tissues. Furthermore, our findings indicated that decreased expression of miR-182 and miR-183 was strongly correlated with poor differentiation, advanced tumor stage, and lymph nodes metastasis ($P<0.05$). Kaplan-Meier survival and log-rank analysis suggested that decreased expression of miR-182 and miR-183 was strongly correlated with shorter overall survival (log-rank test, $P<0.05$). Multivariate Cox proportional hazards model revealed that down-regulated expression of miR-182 and miR-183 were independent prognostic biomarkers of overall survival of NSCLC patients. These findings suggested that down-regulation of miR-182 and miR-183 was associated with the progression and metastasis of NSCLC and would be applied as a therapeutic agent in the treatment of NSCLC.

Keywords: Non-small cell lung cancer, miR-182, miR-183, overall survival, prognosis

Introduction

Lung cancer is currently the most common malignant disease and the leading cause of mortality in the world, and non-small cell lung cancer (NSCLC) accounts for 75-80% of lung cancer cases [1, 2]. Despite the enormous improvements made in chemotherapy and radiotherapy over the past few decades, the 5-year overall survival rate of NSCLC is still less than 15% [3]. The distant metastases are responsible for the failure of NSCLC therapy and the poor prognosis [4]. Therefore, discovery of new specific therapeutic targets may provide effective management for this disease.

MicroRNAs (miRNAs) are a class of small non-coding RNAs that negatively regulate the expression of their target genes by binding to the 3'-untranslated regions (3'-UTRs) of target mRNAs that leads to mRNA degradation or translational suppression [5, 6]. MiRNAs are involved in the regulation of many key biological processes including cell proliferation, differentiation and migration [7]. Mounting evidence revealed that tissue miRNA expression profiles could act as diagnostic or prognostic biomarkers in cancer progression [8, 9].

Dysregulation of different miRNAs have been suggested in term of NSCLC, For example, Guo et al showed that miR-204 was down-regulated in NSCLC tissues and associated with a poor prognosis in NSCLC patients [10]. Xu et al suggested miR-9 was up-regulated in NSCLC tissues and correlated with adverse clinical features and unfavorable survival [11]. In addition, He et al reported that up-regulated expression of miR-452 could inhibit metastasis of NSCLC by regulating BMI1 expression [12]. However,

Table 1. The relationship of miRNAs expression with clinicopathological features of patients with NSCLC

Clinicopathological features	Number	miR-182 expression		miR-183 expression		*P* value (miR-182)	*P* value (miR-183)
		Low	High	Low	High		
Age (years)						0.417	0.892
<60	31	14	17	16	15		
≥60	42	23	19	21	21		
Gender						0.168	0.565
Male	47	21	26	25	22		
Female	26	16	10	12	14		
Tumor size (cm)						0.415	0.127
<3	38	21	17	16	22		
≥3	35	16	19	21	14		
Histologic type						0.897	0.285
Squamous	33	16	17	19	14		
Adenoma	40	20	20	18	22		
Differentiation						0.033	0.002
Mod-well	48	20	28	18	30		
Poor	25	17	8	19	6		
Tumor stage						0.001	0.022
I-II	42	14	28	18	26		
III	31	23	8	21	10		
Lymph nodes metastasis						0.024	0.001
No	52	22	30	20	32		
Yes	21	15	6	17	4		

the role of miRNAs in development and progression of NSCLC remains ambiguous and further studies is needed.

Therefore, our aim is to evaluate the expression pattern of miR-182 and miR-183 in human NSCLC and their association with clinicopathological features.

Materials and methods

Patients and tissue samples

This study was approved by the Research Ethics Committee of Xinxiang Central Hospital. Written informed consent was obtained from all of the patients. All specimens were handled and made anonymous according to the ethical and legal standards.

A total of 73 paired tissue samples of NSCLC and non-tumor tissues were collected from Xinxiang Central Hospital between 2009 and 2010. Patients were underwent surgery without chemotherapy or radiotherapy before the surgery. In term of the tissue samples, the diagnosis was approved by pathologist. All the specimens stored in liquid nitrogen after surgical operation until use. The clinicopathological features are categorized in **Table 1**.

Quantitative real-time PCR

The total RNA isolated from tissues using TRIzol reagent based on the constructor's instructions. Gene specific primers were used to synthesize cDNA from the TaqMan miRNA Assays and reagents from the TaqMan miRNA Reverse Transcription kit (Applied Biosystems). Real-time PCR was carried out to detect the expression level of miRNAs using an invitrogen kit by system of Rotor-gene 6000 (Qiagen). The primers were used from the TaqMan miRNA Assays. The relative amount of miRNAs was normalized with U6 gene as internal reference. The ΔΔCt ($\Delta\Delta Ct = \Delta Ct_{tumor\ samples} - \Delta Ct_{control\ sample}$) to qualify the expression rate of miR-182 and miR-183.

Statistical analysis

All computations were carried out using the software of SPSS version 18.0 for windows. Data were expressed as means ± standard deviation (SD). Differences between groups were evaluated using Student's t-test or χ^2 test. Survival analysis was done by using the log-rank test and Kaplan-Meier method. A Cox proportional hazards model was performed to assess multivariate analyses of prognostic values. $P<0.05$ was statistically significant.

Results

We found that the expression of miR-182 was significantly decreased in NSCLC tissues compared with adjacent non-tumor tissues ($P<0.05$; **Figure 1**), On the other hand, a lower expression of miR-183 was also found in NSCLC tissues when compared with adjacent non-tumor

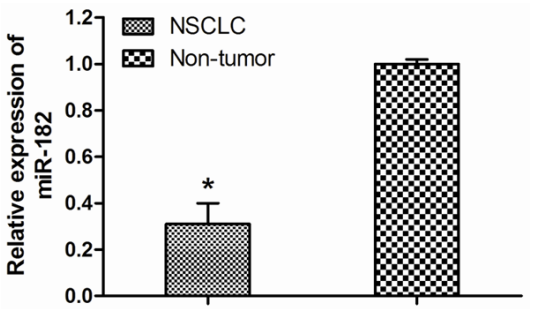

Figure 1. The relative expression level of miR-182 expression between NSCLC tissues and adjacent non-tumor tissues. *P<0.05

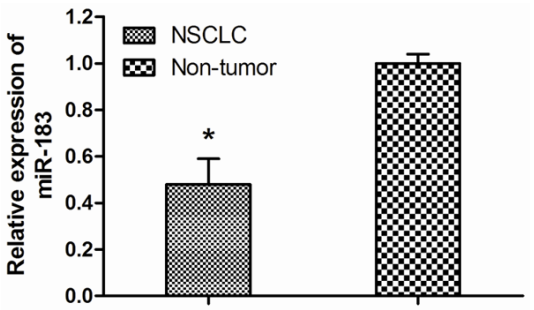

Figure 2. The relative expression level of miR-182 expression between NSCLC tissues and adjacent non-tumor tissues. *P<0.05.

tissues (P<0.05; **Figure 2**). According to the median expression level of miR-182 and miR-183, we divided the patients into low and high expression groups. The correlation between clinicopathological features and miR-182 and miR-183 expression in high and low expression groups were summarized in **Table 1**.

In term of miR-182, our results revealed that down-regulated expression of miR-182 was clearly correlated with poor differentiation, advanced tumor stage, and lymph nodes metastasis (P<0.05). No significant difference was found between miR-182 and age, gender, tumor size, and histologic type (P>0.05) (**Table 1**). In term of miR-183, our data showed that decreased expression of miR-183 was associated with poor differentiation, advanced tumor stage, and lymph nodes metastasis (P< 0.05). There was no significant correlation of miR-183 with other clinicopathological features (**Table 1**).

Kaplan-Meier survival and log-rank analysis were performed to evaluate the association of miR-182 and miR-183 expression with overall survival of NSCLC patients. As shown in **Figures 3** and **4**, the decreased expression of miR-182 and miR-183 was strongly correlated with poor overall survival of NSCLC patients (log-rank test; P<0.05). Multivariate Cox proportional hazards model showed that low expression of miR-182 and miR-183, differentiation, tumor stage, and lymph nodes metastasis were independently associated with poor overall survival of NSCLC patients as prognostic factors (**Tables 2** and **3**).

Discussion

MiRNAs are either tumor suppressors or oncogenes in human carcinogenesis [13]. Dysregulation of miRNAs has been previously reported in many kinds of tumor. For example, Sun et al showed that miR-646 could act as a tumor suppressor and inhibited osteosarcoma cell metastasis by down-regulating FGF2 expression [14]. Dou et al reported that miR-212 could suppress the tumor growth of human hepatocellular carcinoma by targeting FOXA1 [15]. Fujii et al revealed that miR-145 could act as a tumor oncogene and promoted differentiation in human urothelial carcinoma through down-regulation of syndecan-1 expression [16]. Moreover, dysregulation of different miRNAs has been recently suggested in terms of NSCLC. In current study, we evaluated the expression pattern of miR-182 and miR-183 in term of NSCLC.

In the present study, we found that the expression level of miR-182 were significantly decreased in NSCLC tissues compared with adjacent non-tumor tissues, suggesting that miR-182 may function as tumor suppressor in NSCLC progression. Aberrant regulation of miR-182 has been reported in many kinds of malignancies. For example, Sun et al showed that miR-182 was down-regulated in cervical tumor and induced cervical cancer cell apoptosis through inhibiting the expression of DNMT3a [17]. Tang et al reported that miR-182 was down-regulated in human gastric cancer and inhibited gastric cancer cell proliferation through targeting oncogenic ANUBL1 [18]. On the other hand, Zhang et al found that miR-182 was up-regulated in colorectal cancer and promoted cell growth and invasion by targeting forkhead box F2 transcription factor [19]. These studies suggested that miR-182 could be tumor specific and probably dependent on its targets in many kinds of cancer.

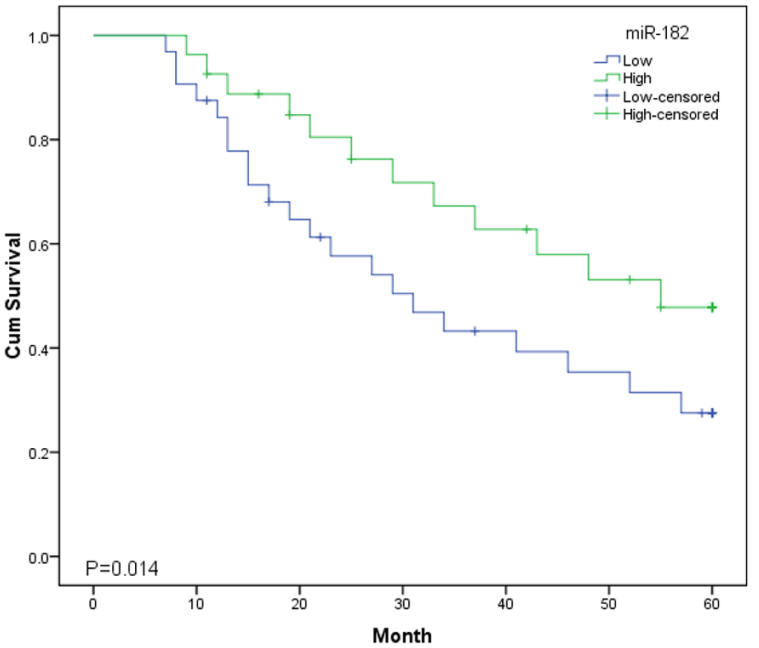

Figure 3. Correlation of miR-182 expression with survival time in patients with NSCLC.

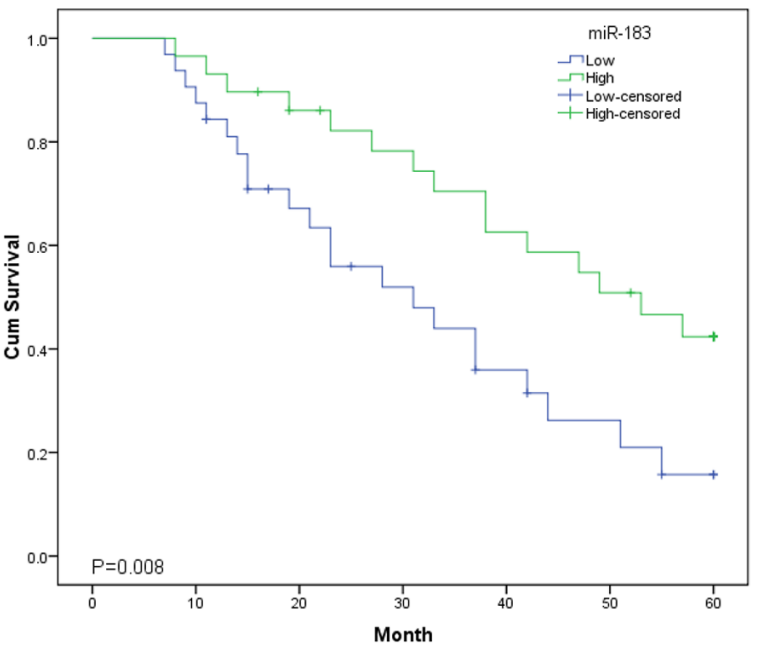

Figure 4. Correlation of miR-183 expression with survival time in patients with NSCLC.

On the other hand, miR-183 expression was down-regulated in NSCLC tissues in comparison with adjacent non-tumor tissues in the current study. It has been reported that miR-183 was down-regulated in many other cancer types. For example, Zhou et al showed that miR-183 was down-regulated in pancreatic ductal adenocarcinoma (PDAC) and associated with advanced clinical features and poor overall survival of PDAC patients. Furthermore, they demonstrated that a low level of miR-183 expression could suppress the growth of PDAC cells via regulation of Bmi-1 expression [20]. Cao et al showed that miR-183 inhibited the invasion of gastric cancer by targeting Ezrin expression [21]. Nevertheless, further investigations were needed to identify the role of miR-183 in the pathogenesis of NSCLC.

Our data showed that low expression of miR-182 and miR-183 was correlated with tumor progression in NSCLC. In term of miR-182, the results indicated that decreased expression of miR-182 was correlated with poor differentiation, advanced tumor stage, and lymph nodes metastasis. In term of miR-183, we found that down-regulated expression of miR-183 was associated poor differentiation, advanced tumor stage, and lymph nodes metastasis. Kaplan-Meier survival and log-rank analysis demonstrated that decreased expression of miR-182 and miR-183 was strongly correlated with shorter overall survival that might be involved in prognosis of NSCLC. Multivariate Cox proportional hazards model showed that low expression of miR-182 and miR-183 may be biomarkers for prognosis in patients that suffered NSCLC.

In conclusion, our result indicated that down-regulated expression of miR-182 and miR-183

Table 2. Multivariate analysis of the correlation of prognosis miR-182 with clinicopathological features

Clinicopathological features	HR	95% CI	*P* value
Age	1.171	0.597-3.184	0.547
Gender	0.717	0.518-1.793	0.687
Tumor size (cm)	1.486	0.762-3.376	0.401
Histologic type	1.215	0.473-2.815	0.524
differentiation	2.149	0.735-5.963	0.029
Tumor stage	2.673	1.078-7.352	0.009
Lymph nodes metastasis	3.471	1.219-9.782	0.004
miR-182	2.817	1.274-9.518	0.003

Abbreviations: HR hazard ratio, 95% CI, 95% confidence interval.

Table 3. Multivariate analysis of the correlation of prognosis miR-183 with clinicopathological features

Clinicopathological features	HR	95% CI	*P* value
Age	1.239	0.631-3.072	0.581
Gender	0.781	0.594-1.872	0.714
Tumor size (cm)	1.573	0.791-3.558	0.424
Histologic type	1.173	0.448-2.715	0.511
differentiation	2.095	0.722-5.839	0.031
Tumor stage	2.815	0.925-7.217	0.011
Lymph nodes metastasis	3.572	1.324-10.037	0.002
miR-183	2.513	1.108-8.392	0.007

Abbreviations: HR hazard ratio, 95% CI, 95% confidence interval.

was associated with progression and metastasis of NSCLC. miR-182 and miR-183 may play key roles in suppression of tumor in NSCLC and would be applied as novel therapeutic agents.

Disclosure of conflict of interest

None.

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