Original Article
Clinical value of microRNA-23a upregulation in non-small cell lung cancer

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Abstract: Background: MiR-23a function as an oncogene in several human cancers, however, its clinical value has not been investigated in NSCLC. Methods: Tissue samples were obtained from 127 NSCLC patients who underwent complete resection at Yantaishan Hospital from March 2008 to January 2014. The expression level of miR-23a was detected in NSCLC tissues and the matched adjacent lung tissues by qRT-PCR. The survival analysis was estimated by the Kaplan-Meier method and was compared by using the log-rank test. Multivariate analysis was performed using the Cox proportional hazard model. Results: The expression level of miR-23a was significantly up-regulated in NSCLC tissues compared with matched adjacent lung tissues (P<0.001). The expression of miR-23a in NSCLC tissues was significantly associated with the smoking status (P=0.001), tumor size (P=0.002), lymphnode metastasis (P<0.001), TNM stage (P=0.001), and tumor differentiation (P=0.004). The overall survival was significantly lower in patients with higher miR-23a expression than in patients with lower miR-23a expression (P=0.02). In addition, multivariate analysis demonstrated that high miR-23a expression (HR=3.558, 95% CI: 2.982-6.635, P=0.011) was significant prognostic factor for NSCLC patients. Conclusions: miR-23a might play an oncogenic role in NSCLC and is a poor prognostic factor. Our results must be verified by large-scale prospective studies with standardized methodology.

Keywords: MicroRNA-23a, expression, NSCLC, prognosis, biomarker

Introduction

Lung cancer is the most common cause of death among all cancers. Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancers [1]. Despite extensive research and significant improvement in early detection and treatment options, the disease is still difficult to treat and many patients develop recurrent diseases after surgery. Therefore, recent studies focused on finding new prognosis biomarker and pivotal molecular associated with development and metastases of NSCLC [2].

MicroRNAs (miRNAs) represent a class of endogenous, highly conserved, small nonprotein-coding RNAs that are approximately 22 nucleotides in length [3]. miRNAs bind to specific sequences in the 3' UTR of target genes, resulting in either modulation of translation efficiency or degradation of the mRNA [4, 5]. miRNAs have been shown to regulate the expression of a variety of genes involved in the carcinogenesis, metastasis, invasion, and recurrence of lung cancer [6-9].

MiR-23a is a miRNA cluster located in chromosome 19p13.12, which can function as an oncogene in several human cancers [10-12]. Previously, Cao et al found that miR-23a regulated TGF-β-induced EMT by targeting E-cadherin in lung cancer cells and might be useful as a new therapeutic target in NSCLC [13]. However, its clinical value has not been investigated in NSCLC.

Materials and methods

Ethics statement

The project protocol was approved by the Institutional Ethics Committee of Yantaishan Hospital prior to the initiation of the study. All patients provided written informed consent for the use of the tumor tissues for clinical research.

Table 1. Correlation of miR-23a expression with clinicopathological characteristics in NSCLC

Characteristic	Number of patients	miR-23a high-expression		miR-23a low-expression		P value
		N	%	N	%	
Age (years)						
≤65	65	34	52.31	31	47.69	0.467
>65	62	31	50.00	31	50.00	
Gender						
Male	77	35	45.45	42	54.55	0.396
Female	50	30	60.00	20	40.00	
Smoking						
Yes	89	54	60.67	35	39.33	0.001
No	38	11	28.95	27	71.05	
Tumor size (cm)						
≤3	41	13	31.71	28	68.29	0.002
>3	86	52	60.47	34	39.53	
Lymphnode metastasis						
Yes	37	29	78.38	8	21.62	<0.001
No	90	36	40.00	54	60.00	
Stage						
I/II	85	35	41.18	50	58.82	0.001
III/IV	42	30	71.43	12	28.57	
Histologicaltype						
Squamous cell carcinoma	64	31	48.44	33	51.56	0.596
Adenocarcinoma	63	34	53.97	29	46.03	
Differentiation						
Well/Moderate	96	42	43.75	54	56.25	0.004
Poor	31	23	74.19	8	25.81	

Patients and tissue samples

Tissue samples were obtained from 127 NSCLC patients who underwent complete resection at Yantaishan Hospital from March 2008 to January 2014. None of the patients had received preoperative radiotherapy or chemotherapy prior to surgical resection. Demographic and clinicopathological data were collected from medical records and histopathological reports. Clinical stage was reassessed according to the 7th edition of the American Joint Committee on Cancer TNM staging system. Follow-up data were included until December 2014 or until death. The clinicopathological characteristics of NSCLC patients are summarized in **Table 1**.

RNA extraction and quantitative RT-PCR

Total RNA was extracted from the corresponding tissues using a miRcute miRNA isolation kit (Tiangen, Bejing, China) according to the manufacturer's instructions. Reverse transcription was performed using the One Step Prime Script miRNA cDNA Synthesis Kit (Takara, Dalian, China) following the manufacturer's instructions. Quantitative RT-PCR was performed using SYBR® Premix Ex Taq™ II (Takara, Dalian, China) according to the manufacturer's instructions with the 7500 real-time RT-PCR system (Applied Biosystems, Foster City). U6B was used as the normalization control. Each detection was performed in triplicate.

Statistical analysis

To evaluate the possible relationships between the expression levels of miR-23a and various clinicopathological parameters, we used either independent sample t test or one-way ANOVA. The survival analysis was estimated by the Kaplan-Meier method and was compared by using the log-rank test. Multivariate analysis
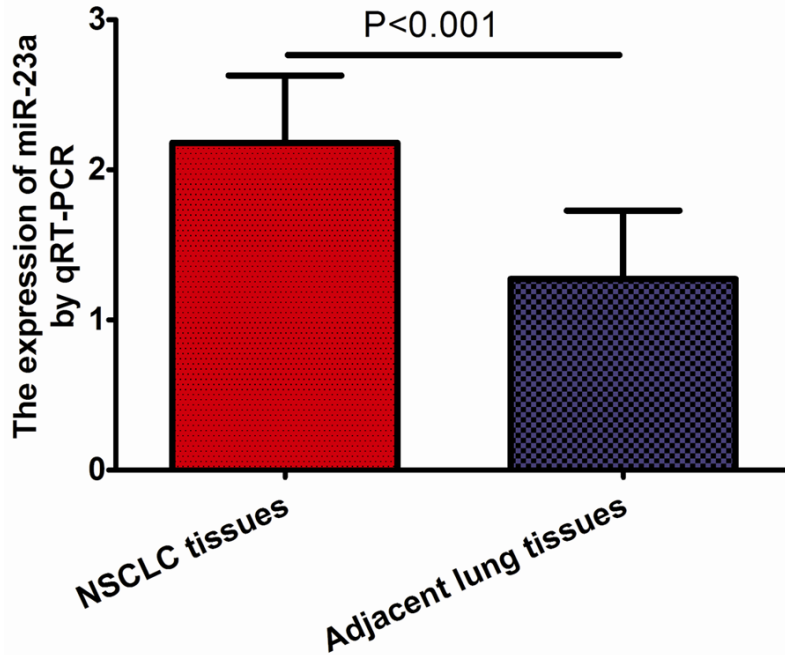
Figure 1. miR-23a expression in 127 NSCLC tissues samples and corresponding normal tissues.
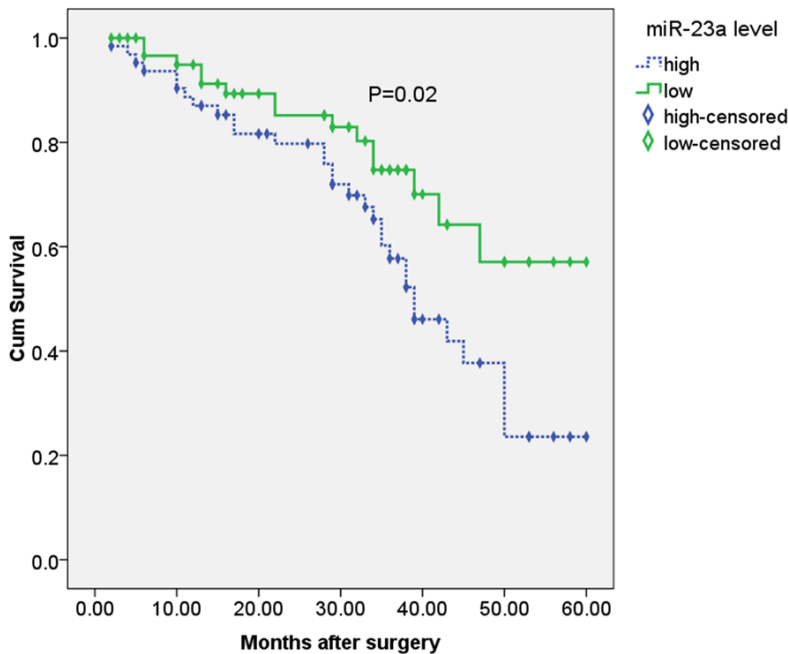
Figure 2. Overall survival of 127 NSCLC patients in relation to mir-23a expression levels.

Results

miR-23a expression in 127 NSCLC tissues samples and corresponding normal tissues

The expression level of miR-23a was detected in 127 pairs of NSCLC tissues and the matched adjacent lung tissues by qRT-PCR. As showed in **Figure 1**, the expression level of miR-23a was significantly up-regulated in NSCLC tissues compared with matched adjacent lung tissues (P<0.001), suggesting that miR-23a takes part in the genesis of NSCLC. The median value of miR-23a expression in NSCLC tissues were used as the cut-off point for the classification of miR-23a-low/high. NSCLC tissues with the relative expression levels of miR-23a exceeding median value were deemed to be miR-23a-high group; all other tissues were considered to be miR-23a-low group.

Correlation of miR-23a expression with clinicopathological characteristics in NSCLC

Of the 127 NSCLC patients, 65 patients were high expression of miR-23a, and 62 patients were low expression of miR-23a. **Table 1** summarized the association of miR-23a upregulation with the clinicopathological features of NSCLC patients. As a result, the expression of miR-23a in NSCLC tissues was significantly associated with smoking status (P=0.001), tumor size (P=0.002), lymph node metastasis (P<0.001), TNM stage (P=0.001), and tumor differentiation (P=0.004).

was performed using the Cox proportional hazard model. A difference was considered significant when P<0.05. All statistical analyses were performed with SPSS version 18.0 (SPSS Inc., Chicago, IL, USA).

Table 2. COX regression analysis in the relationship of clinicopathologic characteristics and prognosis

Characteristics	HR	95% CI	P value
Age (years)	1.371	0.526-3.197	0.381
Gender	0.561	0.228-2.916	0.493
Smoking	2.918	0.816-4.178	0.165
Tumor size (cm)	2.762	0.745-5.197	0.142
Lymphnode metastasis	7.883	2.522-10.047	0.002
Stage	7.912	3.016-10.726	0.001
Histological type	0.273	0.198-3.182	0.372
Differentiation	3.447	1.281-7.883	0.037
miR-23a expression	3.558	2.982-6.635	0.011

High miR-23a level contributes to poor prognosis in patients with NSCLC

Patients with higher miR-23a expression had poorer prognosis. The overall survival was significantly lower in patients with higher miR-23a expression than in patients with lower miR-23a expression (P=0.02, **Figure 2**). In addition, multivariate analysis demonstrated that lymph node metastasis (HR=7.833, 95% CI: 2.522-10.047, P=0.002), tumor stage (HR=7.912, 95% CI: 3.016-10.726, P=0.001), tumor differentiation (HR=3.447, 95% CI: 1.281-7.883, P=0.037), and miR-23a expression (HR=3.558, 95% CI: 2.982-6.635, P=0.011) were significant prognostic factors for NSCLC patients (**Table 2**).

Discussion

Optimal confrontation of NSCLC remains a largely unachieved objective, as the 5-year survival rate struggles to surpass 15% [14]. Most decisions throughout the clinical management of NSCLC are dictated by TNM staging. Surgical removal of tumors, where feasible, is the gold standard of treatment [15]. However, even early-stage resected patients present an extremely variable oncologic outcome; this is a reflection of the large biological heterogeneity between patients who are currently erroneously categorized in the same prognostic group [14]. For example, adjuvant chemotherapy has not been proven to prolong the survival of stage I patients (especially stage IA) [16]. Meanwhile, ~30-40% of stage I NSCLC patients are expected to relapse within 5 years, and this illustrates a severe inability, intrinsic to TNM staging, in defining high-risk patients who should receive adjuvant chemotherapy [17]. In advanced disease stages, an accurate prognostic indicator could guide the administration of maintenance therapy, a clinical decision that currently remains under serious controversy [18]. Consequently, there is a necessity for novel molecular prognostic indicators that would enhance the conventional staging system and improve the decision-making process.

MicroRNAs suppress gene expression by binding to the complementary regions of mRNAs, which either blocks translation or facilitates mRNA degradation through the RNA-induced silencing complex [4]. Recent reports have summarized that aberrant expression of miRNAs has been associated with carcinogenesis and tumor progression [1, 15, 18]. Numerous lines of evidence have demonstrated that miR-23a is involved in the development of various cancers, including colon cancer, hepatocarcinoma, glioma, breast cancer, colorectal cancer, gastric adenocarcinoma, and haematological malignancies [13, 19-25]. Most of them identified miR-23a as an oncogene due to its stimulatory role in cell migration and invasion. For example, miR-23a is regulated by c-MYC and promotes mammary carcinoma cell invasion and hepatic metastasis by targeting Sprouty-2 [23].

The prognostic value of miR-23a has been investigated in several types of cancers. For example, Zhang et al found that miR-23a was significantly up-regulated in laryngeal cancer tissues compared to normal adjacent laryngeal tissues (P<0.01). Tumors with high miR-23a expression had significantly greater extent of lymph node metastasis (P<0.01), worse clinical stage (P<0.05) and shorter overall five-year survival (P<0.01) than those with low miR-23a expression. Both univariate and multivariate Cox hazard regression analysis results showed that clinical stage and miR-23a expression were significantly correlated with patient five-year survival (P<0.01) [12]. He et al identified miR-23a as a tumor suppressor in osteosarcoma. Their data clarify the mechanism of osteosarcoma progression and demonstrated the potential for exploiting miR-23a as a diagnostic marker for osteosarcoma [10]. Ma et al found that the dysregulation of miR-23a and miR-23b might be implicated in the progression of human gastric cancer. Combined expression of miR-23a and miR-23b appeared to be a valu-

able marker for prognosis of gastric cancer [11]. Previously, Cao et al found that miR-23a regulated TGF-β-induced EMT by targeting E-cadherin in lung cancer cells and might be useful as a new therapeutic target in NSCLC [13]. However, its clinical value has not been investigated in NSCLC.

In the present study, we found that the expression level of miR-23a was significantly up-regulated in NSCLC tissues compared with matched adjacent lung tissues, suggesting that miR-23a takes part in the genesis of NSCLC. Of the 127 NSCLC patients, 65 patients were high expression of miR-23a, and 62 patients were low expression of miR-23a. We found that the expression of miR-23a in NSCLC tissues was significantly associated with the smoking status, tumor size, lymph node metastasis, TNM stage, and tumor differentiation. Patients with higher miR-23a expression had poorer prognosis. The overall survival was significantly lower in patients with higher miR-23a expression than in patients with lower miR-23a expression. In addition, multivariate analysis demonstrated that lymph node metastasis, tumor stage, tumor differentiation, and miR-23a expression were significant prognostic factors for NSCLC patients. In conclusion, miR-23a might play an oncogenic role in NSCLC and is a poor prognostic factor. Our results must be verified by large-scale prospective studies with standardized methodology.

Disclosure of conflict of interest

None.

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