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Original Article

MicroRNA-153 expression and prognosis in non-small cell lung cancer

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Abstract: Background: miR-153 has been found to be significantly decreased in non-small cell lung cancer (NSCLC) tissues; however, its clinical significance has not been investigated. Methods: The expression patterns of miR-153 in 137 pairs of human lung cancer tissues and adjacent normal lung tissues were analyzed using qRT-PCR. The relationships between miR-153 expression and clinicopathological parameters were examined by chi-square test. Kaplan-Meier method and the log-rank test were used to determine the difference in overall survival (OS) rates between two groups. Results: The expression of miR-153 was reduced significantly, compared with adjacent normal lung tissues (P<0.05). We observed that the expression level of miR-153 was positively correlated with the clinical stage (P=0.005), lymph node status (P=0.014), distant metastasis (P=0.004), and differentiated degree (P<0.001) in NSCLC patients. According to the Kaplan-Meier survival analysis, the patients with low miR-153 expression exhibited evidently poorer overall survival rates than those with high miR-153 expression (P=0.003). Multivariate analysis showed that the expression of miR-153 was an independent and significant factor associated with poor OS rates (P=0.002). Conclusion: Decreased expression of miR-153 might be a potential unfavorable prognostic factor for patients with NSCLC, and further studies would be needed to prove our findings.

Keywords: NSCLC, microRNA, miR-153, biomarker, prognosis

Introduction

Lung cancer is the most common cancer in the world. Approximately 1.6 million cases of lung cancer have occurred in 2008, of which 80% were non-small cell lung cancer (NSCLC) patients [1]. NSCLC is a slow-developing cancer with a complex pathogenesis; its progression involves several stages as well as activation of many oncogenes and inactivation of tumor suppressor genes.

MicroRNAs (miRNAs) are small non-coding RNAs of 20-22 nucleotides. It represses gene expression through interaction with 3'untranslated regions (3'-UTRs) of mRNAs [2]. miRNAs are predicted to target over 50% of all human protein-coding genes, enabling them to have numerous regulatory roles in many physiological and developmental processes, including development, differentiation, apoptosis and proliferation, through imperfect pairing with target mRNAs of protein-coding genes and the transcriptional or post-transcriptional regulation of their expression [3]. Many miRNAs are deregulated in cancer. They are involved in tumorigenesis and function as oncogenes or tumor suppressor genes [4, 5].

miR-153 has been shown to play an important role in various cancers. However, the effects seem not to be consistent. miR-153 suppresses tumor growth in glioblastoma, epithelial cancer, and leukemia [6-8]. On the contrary, in prostate cancer miR-153 promotes cell proliferation via downregulation of the PTEN tumor suppressor gene [9]. Previously, miR-153 was found to be significantly decreased in lung cancer tissues than the adjacent tissues [10]. However, its clinical significance has not been investigated.

Table 1. Relationship between miR-153 expression and clinicopathologic features in NSCLC

Characteristic	Case number	miR-153 expression		*P* value
		Low (n=69)	High (n=68)	
Age (years)				
<60	53	28	25	0.726
≥60	84	41	43	
Gender				
Male	64	33	31	0.405
Female	83	36	47	
Smoking history				
Yes	107	56	51	0.415
No	30	13	17	
TNM stage				
I+II	104	45	59	0.005
III+IV	33	24	9	
Lymph node status				
Yes	31	22	9	0.014
No	106	47	59	
Distant metastasis				
Yes	12	11	1	0.004
No	125	58	67	
Histological type				
Adenocarcinoma	69	34	35	0.865
Squamous carcinoma	68	35	33	
Differentiated degree				
Low/middle	81	30	51	<0.001
High	56	39	17	

Materials and methods

Patients and samples

137 NSCLC samples resected between March 2007 and April 2013 were retrieved from the Yantaishan Hospital. Before the use of these clinical samples, prior consents from the patients and approval from the local Institutional Ethics Committee were obtained. 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, radiotherapy or immunotherapy prior to the surgery. The clinicopathological data were retrospectively collected by reviewing the patients' medical charts. Patients enrolled in the study were followed to obtain five-year survival data. Survival was defined as the time between the surgery of the primary tumor and mortality or final follow-up of the patient. The clinicopathological data are summarized in **Table 1**.

RNA isolation and quantitative real-time PCR

RNA was extracted from formalin-fixed tissues, using TRIzol reagent, PureLink™ FFPE RNA Isolation Kit and mirVana PARIS kit (Life Technology, California, USA). RNA was diluted in RNase-free water and stored at -80°C before use. For quantitative real-time PCR, the miRNA-specific TaqMan MicroRNA Assays (Applied Biosystems) for miR-153 was used as described by the manufacturer. U6 snRNA was used as an endogenous control for miRNA detection. The expression of miR-153 was quantified by measuring cycle threshold (Ct) values and normalized using the $2^{-\Delta\Delta Ct}$ method relative to U6 snRNA.

Statistical analysis

Data are presented as mean ± SD. The distinct expression of miR-153 between tumor tissues and paracarcinoma tissues was examined by independent samples T-test. The relationships between miR-153 expression and clinicopathological parameters were examined by chi-square test. Overall survival (OS) curves were calculated by the Kaplan-Meier method and the log-rank test was used to determine the difference in OS rates between two groups. $P<0.05$ was considered statistically significant. All the statistical analyses were performed using SPSS18.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

MiR-153 is down-regulated in NSCLC tissues

In order to explore the role of miR-153 in lung carcinogenesis, the expression patterns of miR-153 in 137 pairs of human lung cancer tissues and adjacent normal lung tissues were analyzed using qRT-PCR. The result showed that the expression of miR-153 was reduced significantly, compared with adjacent normal

Table 2. Multivariate Cox's hazards model analysis for prognostic factors

Variable	Hazard ratio	95% CI	*P* value
Sex	1.056	0.378-2.677	0.461
Age	1.728	0.682-3.125	0.287
Smoking history	2.192	0.654-2.182	0.322
Lymph node status	3.127	1.382-8.992	0.009
Distant metastasis	3.925	2.094-10.028	<0.001
TNM stage	3.877	2.987-12.338	<0.001
Histological type	0.553	0.233-1.662	0.762
Differentiated degree	3.172	1.283-4.924	0.041
miR-153 expression level	4.128	2.012-13.221	0.002

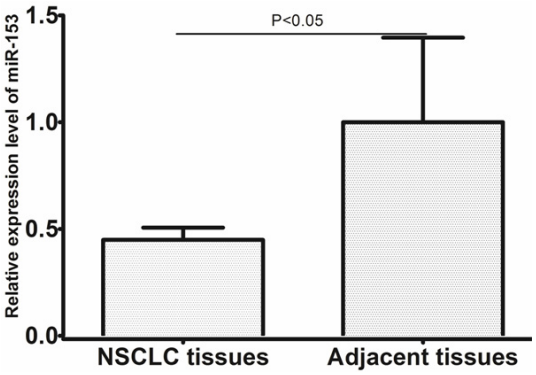

Figure 1. MiR-153 was down-regulated in NSCLC tissues.

lung tissues (shown in **Figure 1**, *P*<0.05). The median value of all 137 lung cancer samples was chosen as the cut-off point for separating tumors with low-level expression of miR-153 from high-level expression miR-153 tumors. Thus, 69 lung cancer patients had low-level expression of miR-153, while 68 lung cancer patients had high-level expression of miR-153.

Relationship between miR-153 expression and clinicopathological characteristics in NSCLC patients

The relationships between miR-153 expression levels and clinicopathological characteristics in individuals with NSCLC are summarized in **Table 1**. We did not find a significant association of miR-153 expression levels with patient's gender (*P*=0.405), age (*P*=0.726), smoking status (*P*=0.415), and histological type (*P*=0.865) in 137 NSCLC cases. However, we observed that the expression level of miR-153 was positively correlated with the clinical stage (*P*=0.005), lymph node status (*P*=0.014), distant metastasis (*P*=0.004), and differentiated degree (*P*<0.001) in NSCLC patients (shown in **Table 1**).

Correlation between miR-153 expression and overall survival

The prognostic value of miR-153 expression for overall survival in NSCLC patients was evaluated by comparing the patients with high and low miR-153 expression. According to the Kaplan-Meier survival analysis, the patients with low miR-153 expression exhibited evidently poorer overall survival rates than those with high miR-153 expression (*P*=0.003; shown in **Figure 2**). The five-year survival rate for patients with low miR-153 expression was 32.3%, compared with 71.6% for patients with high expression. Multivariate analysis was conducted using the Cox proportional hazards model to examine the impact of miR-153 expression and other clinicopathological parameters. The expression of miR-153 emerged as an independent and significant factor associated with poor five-year survival rates (*P*=0.002, shown in **Table 2**).

Discussion

In humans, the miRNAs present in a genome harbor more than 500 experimentally cloned miRNAs, the total number of which could exceed 1000 [11]. Almost 30% of the human genome is estimated to be regulated by miRNAs [12]. A number of reports have demonstrated that miRNAs control development, cell differentiation, apoptosis and proliferation. Moreover, the studies of miRNA expression profiles of human tumors have reported phenotypic signatures of particular cancer types [13, 14]. The prognostic potential of miRNAs has been demonstrated for several types of cancer, including lung cancer. For example, Zhu et al found that miR-224 expression levels were significantly down-regulated in NSCLC compared to the corresponding noncancerous lung tissues. In addition, decreased miR-224 expression was significantly associated with lymph node metastasis, advanced TNM stage, and shorter overall survival. Multivariate regression analysis corroborated that down-regulation of miR-224 was an independent unfavorable prognostic factor for patients with NSCLC [15]. Zhang et al found that miR-10b was significant-

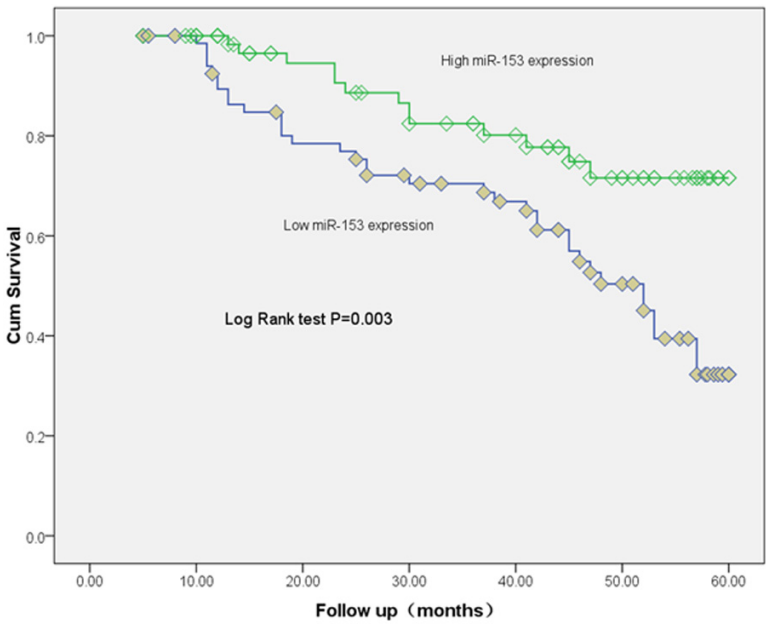

Figure 2. MiR-153 as a prognostic factor in NSCLC patients. Patients with low miR-153 expression had poorer overall survival probability (P<0.05).

ly upregulated in NSCLC tissues as well as in A549 cell line. The relative miR-10b expression levels were significantly positively correlated with TNM stage and regional lymph node involvement. Kaplan-Meier analysis showed that patients with higher levels of miR-10b had significantly poorer survival than those with lower expression of this miRNA in patients, with a 5-year disease-specific survival (DSS) of 29.5 and 63.8%, respectively [16].

Several studies revealed that miR-153 played an important role in various types of cancer. Xu et al found that miR-153 was markedly downregulated in the cells that underwent an epithelial-mesenchy maltransition. Ectopic expression of miR-153 in mesenchymal-like cells resulted in an epithelial morphology change with decreased cellular invasive ability by suppressing SNAI1 and ZEB2 [7]. miR-153 induced apoptosis in a glioblastoma cell line DBTRG-05MG by downregulation of B-cell lymphoma 2 and myeloid cell leukemia sequence 1 [17]. However, there remain some studies showing that miR-153 promotes the development of cancer. Zhang et al. demonstrated that miR-153 upregulation increased colorectal cancer invasiveness and resistance to oxaliplatin and cisplatin both in vitro and in vivo by inducing MMP9 expression [18]. Wu et al found that in human prostate cancer upregulation of miR-153 promotes cell proliferation via downregulation of the PTEN tumor suppressor gene [9]. The opposite activity of miR-153 in various types of cancers may be explained by the diverse biological property of a specific cancer. The role of miR-153 has also been investigated in lung cancer. Yuan et al found that overexpression of miR-153 significantly inhibited the proliferation and migration, and promoted apoptosis of cultured lung cancer cells in vitro, and suppressed the growth of xenograft tumors in vivo. Moreover, miR-153 exerted antitumor activity by targeting protein kinase B (AKT) [10]. Shan et al found that miR-153 inhibited migration and invasion of human NSCLC by targeting ADAM19 [19]. However, the clinical significance of miR-153 has not been investigated.

In the present study, our results showed that the expression of miR-153 was reduced significantly, compared with adjacent normal lung tissues. We did not find a significant association of miR-153 expression levels with patient's gender, age, smoking status, and histological type. However, we observed that the expression level of miR-153 was positively correlated with the clinical stage, lymph node status, distant metastasis, and differentiated degree in NSCLC patients. According to the Kaplan-Meier survival analysis, the patients with low miR-153 expression exhibited evidently poorer overall survival rates than those with high miR-153 expression. The five-year survival rate for patients with low miR-153 expression was 32.3%, compared with 71.6% for patients with high expression. Multivariate analysis was conducted using the Cox proportional hazards model to examine the impact of miR-153 expression and other clinicopathological parameters. The expression of miR-153 emerged as an independent and significant factor associated with poor five-year survival rates.

In conclusion, decreased expression of miR-153 might be a potential unfavorable prognos-

tic factor for patients with NSCLC. Further studies would be needed to prove our findings and to find the role of miR-153 as a creditable clinical predictor for the outcome of NSCLC patients because of the limited sample size of patients in our investigation.

Disclosure of conflict of interest

None.

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