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Original Article

MicroRNA-140-3p expression level is an independent prognostic factor in NSCLC

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Abstract: Objective: The aim of the present study was to explore the clinical significance of microRNA-140-3p expression in NSCLC. Methods: The expression patterns of miR-140-3p in 224 pairs of human lung cancer tissues and normal tissues were analyzed using qRT-PCR. The relationship between clinicopathologic characteristics and miR-140-3p was analyzed by chi-square test. Survival curves were assessed by the Kaplan-Meier method and log-rank tests was used to examine the differences. The Cox proportional hazard regression model was used to analyze the risk factors for NSCLC. Results: The expression of miR-140-3p in NSCLC tissues was lower than that in the adjacent non-tumor tissues. The down-regulation of miR-140-3p was significantly associated with lymphatic invasion ($P = 0.001$), distant metastasis ($P < 0.001$), TNM stage ($P = 0.002$), and tumor grade ($P = 0.025$). Kaplan-Meier analysis with the log-rank test showed that low miR-140-3p expression had a significant impact on OS ($P < 0.001$). Furthermore, Multivariate Cox analysis indicated that miR-140-3p expression was an independent prognostic factor for OS in patients with NSCLC. Conclusion: The miR-140-3p may be a potential prognostic biomarker of NSCLC.

Keywords: MiR-140-3p, NSCLC, prognosis

Introduction

Non small-cell lung cancer (NSCLC), a common type of lung cancer, is one of the most frequently diagnosed types of cancer [1]. Despite recent diagnostic and therapeutic advancements. Limited therapeutic options are available for surgery-resistant NSCLC, resulting in poor prognosis [2, 3]. Early diagnosis and prognostic evaluation of NSCLC are crucial for timely and appropriate treatment.

MiRNAs are 21- to 23-nucleotide, endogenous noncoding RNAs that negatively regulate target gene expressions by binding to its 3'UTR regions [4, 5]. miRNAs play an important role in cancer development and progression by regulating apoptosis and carcinogesis [6, 7]. Moreover, it was reported that miRNAs were correlated with the pathogenesis of many human cancers [8]. For instance, MicroRNA-325-3p upregulation inhibited cell invasion and proliferation by targeting HMGB1 in non-small cell lung cancer [9]. Down-regulation of miR-32 was correlated with NSCLC progression [10]. miRNA-221 promoted human non-small cell lung cancer cell H460 growth by targeting P57 [3]. These results informed that miRNA may be a promising biomarker associated with clinical outcomes in NSCLC.

MiR-140-3p is a newly found miRNA, miR-140-3p was found to inhibit proliferation, migration and invasion of lung cancer cells by targeting ATP6AP2 [11], suggesting that it might function as a tumor suppressor. However, up to date, prognostic role of miR-140-3p expression in NSCLC have not been investigated.

Materials and methods

Patients and samples

This study was approved by the Review Board of Shijitan Hospital, and written informed consent was obtained from all patients. 224 pairs of NSCLC and adjacent tissues were obtained from patients who underwent surgery in hospi-

Table 1. Correlation between miR-140-3p expression and different clinicopathologic features of 224 patients with NSCLC

Parameters	Number of cases	MIR-140-3P EXPRESSION		*P* Value
		Low (n = 116)	High (n = 108)	
Age (y)				0.342
< 60	109	60	49	
≥ 60	115	56	59	
Sex				0.231
Male	111	53	58	
Female	113	63	50	
Smoking History				0.319
Never	30	13	17	
Former + Current	194	103	91	
Histology				0.461
Squamous cell carcinoma	84	40	44	
Adenocarcinoma	89	48	41	
Other	51	28	23	
Lymphatic invasion				0.001
Positive	73	49	24	
Negative	151	67	84	
Distant metastasis				< 0.000
Positive	70	53	17	
Negative	154	63	91	
TNM stage				0.002
I/II	81	31	50	
III/IV	143	85	58	
Tumor grade				0.025
I-II	105	46	59	
III	119	70	49	

tal from 2007 to 2015. None of patients had received chemotherapy, immunotherapy and radiotherapy prior to surgery. Immediately following surgical resection, the tissue samples were immediately frozen in liquid nitrogen and stored at -80°C until further analysis. Patient characteristics are shown in **Table 1**.

Quantitative real-time PCR

Total RNA was extracted from frozen samples using Trizol reagent (Invitrogen, Pudong, Shanghai, China) according to the manufacturer's instructions. The RNA concentration was measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). The expression of miR-140-3p was determined by miScript SYBR Green PCR Kit (Qiagen), using real-time PCR. Expression of miR-101 was normalized according to the internal RNU6B control, and plotted as relative value, which were calculated by using the 2-ΔΔCT method. Each sample was examined in triplicate for qRTPCR.

Statistical analysis

The comparison of the expression levels of miR-140-3p between NSCLC tissues and adjacent normal tissues were performed using the two-sample Student's t test. The chi square or Fisher's exact probability test was used to examine possible association between miR-140-3p expression and clinicopathologic factors. Survival analysis was conducted with the Kaplan-Meier method. Multivariate analysis of the prognostic factors was performed with Cox regression model. Significant differences were accepted at $P < 0.05$. All statistical analyses were performed with SPSS version 18.0 (SPSS Inc., Chicago, IL, USA).

Results

miR-140-3p is downregulated in human lung cancer tissues

miR-140-3p expression was detected in 224 pairs of NSCLC and corresponding adjacent noncancerous tissues normalized to U6 small nuclear RNA. As shown in **Figure 1**, Relative miR-140-3p level was found to be significantly lower in patients with NSCLC than healthy controls ($P < 0.01$).

miR-140-3p downregulation associates with advanced clinicopathological features of human lung cancer

For better understanding of the potential roles of miR-140-3p in NSCLC development and progression, patients were divided into two groups (high and low) according to the median level of relative quantity. The relationships of the miR-140-3p with various clinical factors of NSCLC were analyzed and summarized in **Table 1**. Low expression of miR-140-3p was found to significantly correlate with lymphatic invasion

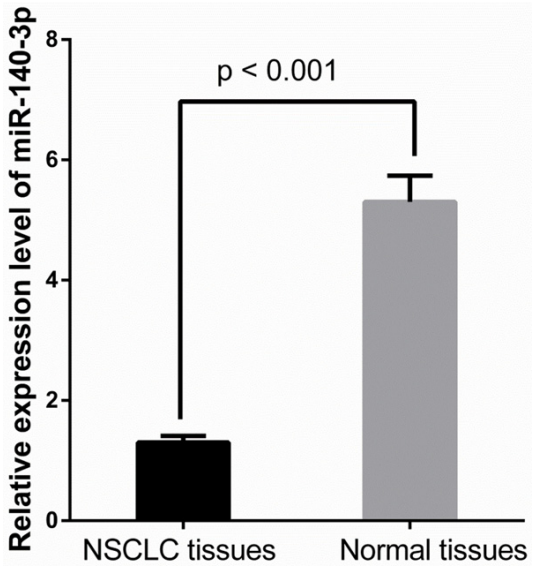

Figure 1. Expression level of the miR-140-3p in NSCLC tissues and matched normal tissues.

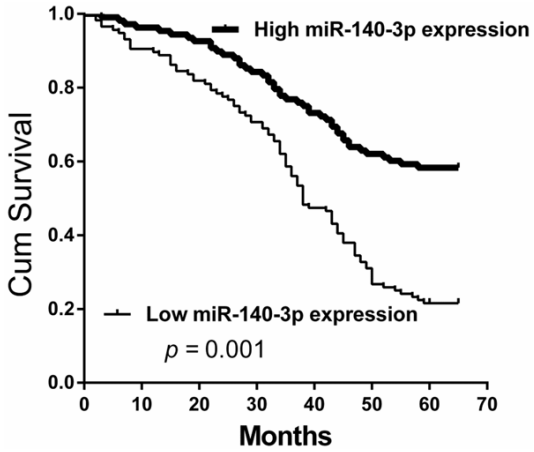

Figure 2. Kaplan-Meier survival curves of OS based on miR-140-3p expression.

(P = 0.001), distant metastasis (P < 0.000), TNM stage (P = 0.002), and tumor grade (P = 0.026). However, we did not find any significant correlation between miR-198 levels and other clinicopathological features.

Low-expression level of miR-140-3p predicts poor prognosis in NSCLC patients

Using the Kaplan-Meier method and log-rank test, the overall survival of patients with low miR-140-3p expression levels were found to be significantly shorter than those of patients with high miR-140-3p expression levels (P = 0.001; **Figure 2**). Furthermore, we performed univariate and multivariate analysis using the Cox propotional harzard regression model to determine whether miR-140-3p expression and other clinical parameters are independent factors for prognostic prediction in NSCLC patients. Multivariate analysis showed that miR-140-3p expression level was independent prognostic factors for overall survival (P = 0.013) shown in **Table 2**.

Discussion

Lung cancer is the leading cause of cancer-related death worldwide [12]. Even if the recent development in tumor diagnosis and treatment have been tremendous, the outcome of the patients with NSCLC remains poor [13, 14]. It is of great significance to explore the underlining molecular mechanisms and to identify a powerful prognostic indicator for human lung cancer. Several clinical prognostic markers for lung cancer have been recognized to dichotomize the risk, such as age, sex, tumor stage [15]. However, the sensitivity of those biomarkers is not satisfied. Therefore, it is imperative to develop novel sensitive and specific biomarkers for detection of NSCLC. MicroRNA-140-3p is a new discovered miRNA. there is little research about the role of miR-140-3p in progression of tumor. Recently, Kong et al. found that MicroRNA-140-3p functioned as atumor suppressor by targeting ATP6AP2 [11]. However, the prognostic value of miR-140-3p in NSCLC was not reported.

In our present study, we found that miR-140-3p was downregulated in NSCLC tissues in comparison with matched noncancerous lung tissues. To explore the association between the miR-140-3p expression levels and the clinicopathological factors. patients were divided into two groups (high and low). Our results showed that The associations between the miR-140-3p expression level and lymphatic invasion, distant metastasis, tumor grade and TNM stage were significant. The result of Kaplan Meier analysis revealed that Low miR-140-3p levels in NSCLC were associated with shorter OS (P = 0.001). Furthermore, multivariate analysis indicated miR-105 as an independent prognostic indicator for glioma patients (P = 0.013). Therefore, our finding suggested that miR-140-3p could be an potential factor that plays an

Table 2. Multivariate analysis of prognostic factors in patients with NSCLC

Variables	Hazard ratio	95% CI	*P* value
Age	1.729	0.412-4.337	0.517
Sex	0.721	0.335-2.518	0.612
Smoking History	2.736	0.717-4.398	0.256
Histology	1.313	0.422-2.641	0.489
Lymphatic invasion	3.327	2.212-10.137	0.019
Distant metastasis	4.818	3.337-9.973	0.006
TNM stage	4.467	2.781-11.456	0.018
Tumor grade	3.441	1.782-10.885	0.039
miR-140-3p expression	4.379	2.541-10.337	0.013

important role in the malignant progression of gliomas.

MiR-140-3p has been observed to be involved in multiple physiological and pathological processes. For example, Song et al. found that up-expression of miR-140 inhibited cell migration and invasion in both osteosarcoma and colon cancer cell lines [16]. More recently, Yang et al. reported that miR-140-5p inhibits migration and invasion in HCC cell lines by targeting transforming growth factor β receptor 1 and fibroblast growth factor 9 [17]. Furthermore, Yuan et al. identified that miR-140-3p inhibits tumor progression by targeting Insulin-Like Growth Factor 1 Receptor (IGF1R) in NSCLC [18]. Similarly, Kong et al. found that microRNA-140-3p inhibits proliferation, migration and invasion of lung cancer cells by targeting ATP6AP2 [11]. Those results informed that miR-140-3p expression may be correlated with tumor progression and function as tumor suppressor.

Conclusion

To our knowledge, this is the first study on clinical significance of levels of miR-140-3p in NSCLC patients. Our study revealed that the expression levels of miR-140-3p were down-regulated in NSCLC tissues. Low expression of miR-140-3p was correlated with poor prognosis for NSCLC. MiR-140-3p may be useful for evaluating prognosis and may provide a novel target for the treatment of patients with NSCLC.

Disclosure of conflict of interest

None.

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References

[1] Jemal A, Siegel R, Xu J and Ward E. Cancer statistics, 2010. CA Cancer J Clin 2010; 60: 277-300.

[2] Gazala S, Pelletier JS, Storie D, Johnson JA, Kutsogiannis DJ and Bedard EL. A systematic review and meta-analysis to assess patient-reported outcomes after lung cancer surgery. ScientificWorldJournal 2013; 2013: 789625.

[3] Xu Y, Zhong C, Ding S, Huang H and Shen Z. MicroRNA-221 promotes human non-small cell lung cancer cell H460 growth. Int J Clin Exp Med 2015; 8: 2024-2030.

[4] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.

[5] Zhang B, Pan X, Cobb GP and Anderson TA. microRNAs as oncogenes and tumor suppressors. Dev Biol 2007; 302: 1-12.

[6] Galluzzi L, Senovilla L, Vitale I, Michels J, Martins I, Kepp O, Castedo M and Kroemer G. Molecular mechanisms of cisplatin resistance. Oncogene 2012; 31: 1869-1883.

[7] Barroso-delJesus A, Romero-Lopez C, Lucena-Aguilar G, Melen GJ, Sanchez L, Ligero G, Berzal-Herranz A and Menendez P. Embryonic stem cell-specific miR302-367 cluster: human gene structure and functional characterization of its core promoter. Mol Cell Biol 2008; 28: 6609-6619.

[8] Yin QW, Sun XF, Yang GT, Li XB, Wu MS and Zhao J. Increased expression of microRNA-150 is associated with poor prognosis in non-small cell lung cancer. Int J Clin Exp Pathol 2015; 8: 842-846.

[9] Yao S, Zhao T and Jin H. Expression of MicroRNA-325-3p and its potential functions by targeting HMGB1 in non-small cell lung cancer. Biomed Pharmacother 2015; 70: 72-79.

[10] Bai Y, Wang YL, Yao WJ, Guo L, Xi HF, Li SY and Zhao BS. Expression of miR-32 in human non-small cell lung cancer and its correlation with tumor progression and patient survival. Int J Clin Exp Pathol 2015; 8: 824-829.

[11] Kong XM, Zhang GH, Huo YK, Zhao XH, Cao DW, Guo SF, Li AM and Zhang XR. MicroRNA-140-3p inhibits proliferation, migration and invasion of lung cancer cells by targeting ATP6AP2. Int J Clin Exp Pathol 2015; 8: 12845-12852.

[12] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[13] Liu L, Qu W and Zhong Z. Down-regulation of miR-503 expression predicate advanced mythological features and poor prognosis in patients with NSCLC. Int J Clin Exp Pathol 2015; 8: 5609-5613.
[14] Kocaturk CI, Gunluoglu MZ, Cansever L, Demir A, Cinar U, Dincer SI and Bedirhan MA. Survival and prognostic factors in surgically resected synchronous multiple primary lung cancers. Eur J Cardiothorac Surg 2011; 39: 160-166.
[15] Qu WQ, Liu L and Yu Z. Clinical value of microRNA-23a upregulation in non-small cell lung cancer. Int J Clin Exp Med 2015; 8: 13598-13603.
[16] Song B, Wang Y, Xi Y, Kudo K, Bruheim S, Botchkina GI, Gavin E, Wan Y, Formentini A, Kornmann M, Fodstad O and Ju J. Mechanism of chemoresistance mediated by miR-140 in human osteosarcoma and colon cancer cells. Oncogene 2009; 28: 4065-4074.
[17] Yang H, Fang F, Chang R and Yang L. MicroRNA-140-5p suppresses tumor growth and metastasis by targeting transforming growth factor beta receptor 1 and fibroblast growth factor 9 in hepatocellular carcinoma. Hepatology 2013; 58: 205-217.
[18] Yuan Y, Shen Y, Xue L and Fan H. miR-140 suppresses tumor growth and metastasis of non-small cell lung cancer by targeting insulin-like growth factor 1 receptor. PLoS One 2013; 8: e73604.