Clinical Research Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
2019, Vol. 47(2) 867–874

DOI: 10.1177/0300060518815379
journals.sagepub.com/home/imr
SAGE

Expression and clinical significance of miR-139-5p in non-small cell lung cancer

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Abstract

Objective: MiR-139-5p is a common tumor-associated microRNA (miRNA), which inhibits the occurrence and development of malignant tumors from various tissue sources. We detected miR-139-5p expression levels in tissues from patients with non-small cell lung cancer (NSCLC) to explore the relationship between miR-139-5p expression and clinicopathological parameters.

Methods: MiR-139-5p expression levels were detected in cancerous and normal tissues from 60 NSCLC patients by fluorescence quantitative polymerase chain reaction, using normal paracancerous tissue as a control. The relationships between miR-139-5p and clinicopathological parameters of NSCLC, including survival, were analyzed by *t*-tests and univariate analysis.

Results: MiR-139-5p expression levels were significantly reduced in NSCLC tissues compared with normal adjacent tissue. MiR-139-5p expression was not significantly associated with age, sex, or smoking history, but was related to clinical stage, pathological type, tumor size, and lymph node metastasis. Furthermore, low expression of miR-139-5p, clinical stage (II/III), adenocarcinoma, tumor $\geq$3 cm, and lymph node metastasis were all related to overall survival.

Conclusion: MiR-139-5p expression levels are down-regulated in NSCLC tissues, and low expression is associated with clinical stage, pathological type, tumor size, and lymph node metastasis in NSCLC patients. MiR-139-5p may act as a tumor suppressor gene in the occurrence and development of NSCLC.

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Keywords
Non-small cell lung cancer, miR-139-5p, miRNA, fluorescence quantitative PCR, tumor suppressor gene, clinical stage, pathological type, tumor size, lymph node metastasis, survival

Date received: 12 July 2018; accepted: 2 November 2018

Introduction

Lung cancer is the most common malignant tumor worldwide, and non-small cell lung cancer (NSCLC) is the main pathological type of lung cancer; however, there are currently no specific treatment methods for NSCLC.[1,2] Although the application of new biological treatment methods, such as immunotherapy and targeted therapy, and the rapid development of multidisciplinary treatments including surgery, radiotherapy, and chemotherapy, have greatly improved the therapeutic effects and improved survival in patients with NSCLC, the death rate remains high. There is thus an urgent need to develop novel therapeutic methods and drugs.

MicroRNAs (miRNAs) are endogenous non-coding small RNA molecules, which affect the expression of their target genes through post-transcriptional regulation, thus regulating cell proliferation, differentiation, and apoptosis.[3–5] Increasing evidence has implicated miRNAs in the occurrence and development of malignant tumors, including NSCLC,[6,7] though their clinical application in the diagnosis and treatment of NSCLC has yet to be evaluated. MiR-139-5p is an miRNA of 18–22 nucleotides located in the human chromosome 11q13.4 region. Mir-139-5p has been identified as a common tumor-associated miRNA,[8,9] and several studies have revealed that it may play different roles in malignant tumors derived from different tissue sources. Wang et al.[10] revealed that miR-139-5p expression in the peripheral blood was significantly increased in patients with prostate cancer compared with healthy volunteers. Furthermore, expression levels of miR-139-5p in peripheral blood were significantly higher in prostate cancer patients with high differentiation, metastatic carcinoma, and high-risk Gleason score compared with patients with low differentiation, *in situ* cancer, and intermediate-risk Gleason score. These results suggested that miR-139-5p may play an oncogenic role in the development and progression of prostate cancer. However, other researchers have found conflicting results in studies of malignant tumors from other tissue sources. According to Cao et al.,[11] reduced miR-139-5p expression in *KRAS* mutant colorectal cancer cells was significantly correlated with proliferation, migration, and invasion of colon cancer cells. Other researchers[12] found that miR-139-5p expression levels were significantly decreased in gastric cancer tissues, and low expression was related to lymph node metastasis and high TNM stage in gastric cancer patients. However, studies of miR-139-5p in NSCLC are currently lacking.

We therefore detected the expression levels of miR-139-5p in 60 cancerous and matched paracancerous tissue samples from NSCLC patients using fluorescence quantitative polymerase chain reaction (PCR). We analyzed the relationship between miR-139-5p expression and clinicopathological parameters, and investigated the feasibility of miR-139-5p as an effective target and prognostic factor for the diagnosis and treatment of NSCLC.

Materials and methods

Patients

NSCLC tissues and corresponding paracancerous tissue samples were randomly collected from 60 NSCLC patients undergoing surgical excision at the Department of Cardiothoracic Surgery, Yangtze University Hospital, from March 2013 to March 2018. This study was approved by the Ethics Committee of the Affiliated Hospital of Yangtze University. The patients and/or their families signed informed consent after being informed about the use of the cancerous and paracancerous tissue samples.

The inclusion criteria were patients diagnosed with NSCLC by pathological examination, who underwent tumor resection and who had not received radiochemotherapy or other immunotherapy before surgery, and for whom there was at least 3 months of comprehensive follow-up data. Patients were excluded if they had distant tumor metastases or malignant pleural effusions, a previous history of cancer, severe cardiovascular, lung, kidney, or other diseases that could seriously affect survival time, or if there was insufficient clinical data.

Collection of tissue samples. Tumor tissues were collected from the 60 patients during surgery and NSCLC was confirmed by pathological diagnosis. Normal paracancerous tissues were collected from the same patients as a control. The collection of the tissues was completed within 30 minutes *in vitro*. Cancerous samples approximately $1.5\,cm \times 1.0\,cm \times 1.0\,cm$ were collected from the non-necrotic zone of the longitudinal section of the primary tumor tissue, taking care to avoid including any non-cancerous tissue. Paracancerous tissue samples were collected from normal tissues 2 cm away from the tumor edge, and were then sectioned and examined by pathologists to confirm the absence of tumor cells. All the tissue samples collected as above were isolated and placed immediately in a freezer at −80°C for later use.

RNA extraction. The collected tissue blocks were homogenized and shaken in 500 μL Trizol (Sangon Biotech Co., Ltd., Shanghai, China), according to the instructions of the fluorescence quantitative miR-139-5p system (Guangzhou Ribo Co., Ltd., Guangzhou, China), followed by the addition of 170 μL chloroform and centrifugation at 4°C at $12{,}879 \times g$ for 10 minutes. Absolute ethyl alcohol 500 μL was then added to the supernatant and mixed thoroughly, 700 μL of the resulting mixture was centrifuged at $8{,}944 \times g$ at room temperature for 15 seconds, and 50 μL of RNase-free water was added to elute the attached RNA. The centrifugation product was stored at −80°C for later use. The concentration, purity, and amount of total RNA were measured using an ultra-micro UV nucleic acid analyzer (Thermo Fisher Scientific (China) Inc., Shanghai, China).

Reverse transcription-PCR. The prepared reaction fluid was placed on ice and 2 μL of total RNA was reversed transcribed into cDNA at 37°C for 60 minutes, followed by 85°C for 5 minutes. The resulting cDNA was diluted 1:5–1:10 with RNase-free water for further testing.

PCR amplification and fluorescence detection were carried out using a real-time fluorescence quantitative PCR system (ABI, Thermo Fisher Scientific, Waltham, MA, USA) under the following reaction conditions: 95°C for 10 minutes, 92°C for 15 seconds, and 60°C for 1 minute, for 40 cycles. After the end of the PCR reaction, the amplification curves and melting curves were produced by using the real-time fluorescence quantitative PCR system's own software. The content of miR-139-5p in each sample and its

expression relative to the internal reference gene U6snRNA in the same sample were calculated by the $2^{-\Delta\Delta Ct}$ method, where $\Delta\Delta Ct$ was the relative expression of miR-139-5p.

Statistical analysis. The experimental data were analyzed using SPSS version 19.0 (IBM Corp., Armonk, NY, USA). All results were expressed as mean ± standard deviation. Mean values were compared between two groups by independent *t*-test, and factors influencing overall survival (OS) among NSCLC patients were analyzed by Cox proportional hazards regression. We also calculated odds ratios and 95% confidence intervals. $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

There were 38 male (63.3%) and 22 female patients (36.7%), with a mean age of 56.5 years (range 41 to 78 years). The median follow-up time was 45 months (range 3 to 60 months).

MiR-139-5p expression

MiR-139-5p expression levels were compared between 60 cancerous and 60 corresponding normal paracancerous tissue samples from NSCLC patients using fluorescence quantitative PCR. The obtained melting curves (Figure 1) confirmed that the distributions of miR-139-5p and the reference gene U6 were unimodal, indicating no primer dimers and no non-specific amplification. The results were therefore considered reliable. Fluorescence quantitative PCR (Figure 2) showed that the expression level of miR-139-5p was significantly lower in NSCLC tissue compared with normal paracancerous tissue ($P < 0.01$).

MiR-139-5p expression in relation to clinicopathological parameters

The NSCLC patients were classified according to clinicopathological parameters including age, sex, clinical stage, pathological type, tumor size, lymph node metastasis, and smoking history, and miR-139-5p expression levels in cancerous tissues were compared between the respective groups. As shown in Table 1, miR-139-5p expression was not significantly associated with age, sex, or smoking history, but expression levels were significantly lower in patients with high clinical stage, adenocarcinoma, tumor ≥ 3 cm, or lymph node metastasis compared with patients with low clinical stage, squamous carcinoma, tumor < 3 cm, or no lymph node metastasis, respectively ($P < 0.05$).

Relationships between miR-139-5p expression and clinicopathological parameters and survival

We analyzed the correlations between age, sex, clinical stage, pathological type, tumor size, lymph node metastasis, smoking history, and miR-139-5p expression and OS among NSCLC patients by univariate Cox regression analysis. Low expression of miR-139-5p, clinical stage (II/III), adenocarcinoma, tumor ≥ 3 cm, and lymph node metastasis were identified as risk factors influencing OS (Table 2).

Discussion

MiRNAs have demonstrated close relationships with the occurrence and development of malignant tumors.[8] MiRNAs can regulate the relevant signal transduction pathways in cells, with abnormal expression potentially causing the cells to become malignant and eventually leading to tumorigenesis. However, the biological functions of miRNAs have been suggested to vary

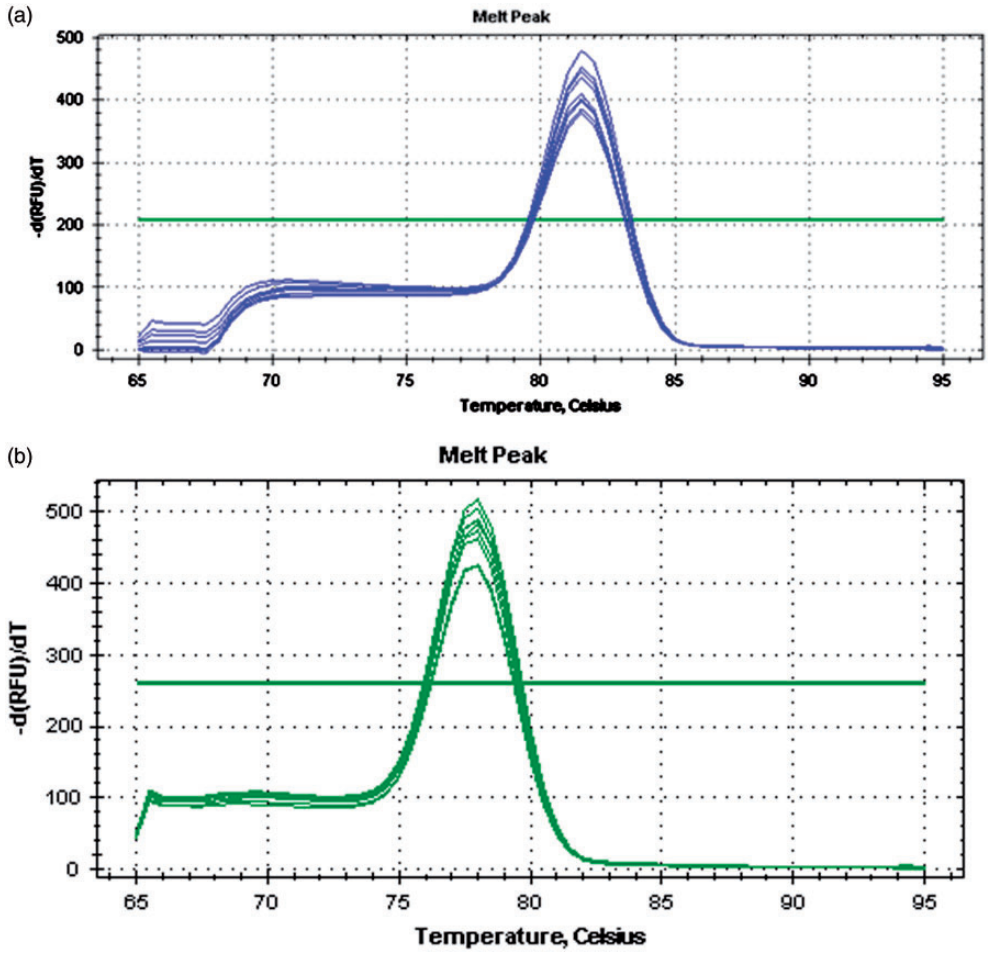

Figure 1. Melting curves of (a) miR-139-5p and (b) U6.

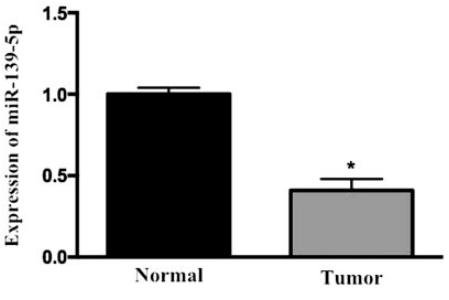

Figure 2. MiR-139-5p expression levels in normal and tumor tissue samples. *$P < 0.01$ vs normal tissue.

depending on the target genes and tissue sources.[13–17] Some miRNAs are considered as biomarkers for the prognosis of diseases, including malignant tumors, and the detection of expression levels of relevant miRNAs may thus help in tumor diagnosis and prognosis prediction.

Various miRNAs have demonstrated abnormal expression levels in NSCLC tissues, among which miR-34a, miR-204, miR-186, and miR-134 were down-regulated[18–21] and miR-21, miR-221, miR-205,

Table 1. MiR-139-5p expression level in relation to different clinicopathological parameters.

Clinicopathological parameter	Number of cases	MiR-139-5p expression	*t* value	*P* value
Sex				
Male	38	0.45 ± 0.04	0.918	0.422
Female	22	0.41 ± 0.05		
Age (years)				
<50	35	0.43 ± 0.06	0.109	0.514
≥ 50	25	0.47 ± 0.03		
Pathological type				
Squamous carcinoma	28	0.65 ± 0.05	2.186	0.032
Adenocarcinoma	32	0.37 ± 0.07		
Clinical stage				
Stage I	29	0.71 ± 0.05	4.29	0.021
Stage II–III	31	0.33 ± 0.06		
Size of tumor (cm)				
≥ 3	19	0.68 ± 0.08	1.208	0.041
<3	41	0.41 ± 0.39		
Smoking history				
Smoking	22	0.41 ± 0.07	0.461	0.164
Not smoking	38	0.48 ± 0.05		
Lymph node metastasis				
Yes	17	0.23 ± 0.05	1.378	0.031
No	33	0.66 ± 0.04		

Table 2. Univariate analysis of miR-139-5p and clinicopathological features in relation to overall survival in NSCLC patients.

Clinicopathological parameter	β	Standard deviation	*P* value	OR (95% CI)
Sex	0.378	0.322	0.365	1.68 (0.42–2.37)
Age	0.208	0.144	0.491	1.23 (0.70–1.81)
Clinical stage	0.673	0.346	0.040	2.03 (1.16–2.70)
Pathological type	0.987	1.513	0.037	2.77 (1.79–5.24)
Size of tumor	0.408	0.448	0.041	1.54 (1.01–2.14)
Smoking history	0.215	0.217	0.350	1.28 (0.48–1.82)
Lymph node metastasis	0.322	0.177	0.025	1.03 (0.59–1.42)
Low miR-139-5p	1.351	3.618	0.033	3.12 (1.69–6.11)

OR, odds ratio; CI, confidence interval.

and miR-638 were up-regulated.[22–25] We aimed to identify the role of miR-139-5p in the development of NSCLC and the progression of malignancy by comparing its expression levels in 60 cancerous and paracancerous tissue samples from NSCLC patients. The results showed that miR-139-5p expression was significantly lower in NSCLC tissues compared with normal adjacent tissues ($P < 0.01$), suggesting that down-regulation of miR-139-5p expression might play a key role in the development of NSCLC. We also reviewed the clinical data of the same 60 patients and compared miR-139-5p expression levels between patients grouped according to certain

clinicopathological parameters. The result showed that miR-139-5p expression was unaffected by age, sex, or smoking history, but was significantly lower in patients with high clinical stage, adenocarcinoma, tumor $\geq$3 cm, and lymph node metastasis compared with patients without these features. Furthermore, low expression of miR-139-5p was identified as a risk factor for shorter OS in NSCLC patients with clinical stage (II/III), adenocarcinoma, or tumor $\geq$3 cm, determined by univariate Cox regression analysis. Liu et al.[26] analyzed the expression of miR-139-5p in small cell lung cancer and showed that expression levels were significantly lower in small cell lung cancer tissues compared with normal paracancerous tissues. Furthermore, miR-139-5p expression was not related to patient sex or age, but was closely related to the stage of disease, sensitivity to chemotherapy, and OS, while Cox regression analysis found that staging of small cell lung cancer and miR-139-5p expression were independent prognostic factors. These results were consistent with the results of the current study, indicating that miR-139-5p expression is reduced in malignant tumors derived from lung tissue, and suggesting that it might act as a tumor suppressor gene in this situation.

Overall, the findings of this study suggest that miR-139-5p expression was abnormally down-regulated in NSCLC tissues, and that low expression of miR-139-5p was associated with clinical stage, pathological type, tumor size, and lymph node metastasis in NSCLC patients. We therefore speculated that miR-139-5p may act as tumor suppressor gene during the occurrence and development of NSCLC; however, further studies are needed to clarify the specific mechanism of miR-139-5p in NSCLC.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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