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The expression and clinical significance of miRNA-99a and miRNA-224 in non-small cell lung cancer

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Abstract. – OBJECTIVE: To investigate the expression and clinical significance of miRNA-99a and miRNA-224 in the serum of patients with non-small cell lung cancer (NSCLC).

PATIENTS AND METHODS: 83 patients with NSCLC, who were diagnosed and treated in our hospital from January 2014 to September 2017, were included in the experiment group. 79 patients, who made health check up, were included in the control group. The quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) technique was used to test the expressions of miRNA-99a and miRNA-224 in the serum of the patients in the two groups, and the relationship between the expression levels of miRNA-99a and miRNA-224 and the clinicopathological features of the patients with NSCLC was analyzed; the correlation between the expression of miRNA-99a and the expression of miRNA-224 in NSCLC was also analyzed.

RESULTS: The expression level of miRNA-99a in the patients with NSCLC was significantly lower than that in the patients in the normal control group; the differences were statistically significant ($p<0.001$). The expression level of miRNA-224 in the patients with NSCLC was markedly higher than that in the patients in the normal control group; the differences were statistically significant ($p<0.001$). The expression level of miRNA-99a in the patients with NSCLC was remarkably correlated with pathological stage, the presence or absence of lymph node metastasis and tissue differentiation ($p<0.001$). The expression level of miRNA-224 in the patients with NSCLC was significantly correlated with pathological stage, the presence or absence of lymph node metastasis and pathological grade ($p<0.001$). The results of partial correlation analysis showed that the expression levels of miRNA-99a and miRNA-224 were negatively correlated with each other in NSCLC ($r=-0.985$, $p<0.001$).

CONCLUSIONS: MiRNA-99a and miRNA-224 may be involved in the development and progression of NSCLC. MiRNA-99a is associated with NSCLC pathological stage, lymph node metastasis and tissue differentiation, while miRNA-224 is associated with NSCLC pathological stage, lymph node metastasis and pathological grade. MiRNA-99a and miRNA-224 can be used as clinical monitoring indicators for NSCLC.

Key Words:
MiRNA-99a, MiRNA-224, Non-small cell lung cancer, Expression, Clinical significance.

Introduction

Lung cancer is one of the most common malignant tumors in the respiratory system, and according to the histological type of lung cancer, the World Health Organization classifies lung cancer into adenocarcinoma, small cell carcinoma, squamous carcinoma and adenosquamous carcinoma[1,2]. According to statistics, the morbidity of cancers in the world in 2012 was 141 million, while the morbidity of lung cancer was 16 million; it accounted for 12.9% of the morbidity of global cancers[3]. In recent years, people have conducted in-depth studies on lung cancer, but the early diagnosis and survival rate of patients with lung cancer have not been significantly improved. Many patients with lung cancer have been in advanced stage once they are diagnosed, and the treatment and prognosis of advanced lung cancer are poor; the five-year survival rate is less than 15%, which will seriously threaten the safety and health of human life[4,5].

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MicroRNAs, as members of the regulation network of genes, are proved to play a very important role in the incidence and development of lung cancer and other various tumors in many studies, which also provide new clues for the study of lung cancer[6,7]. MiRNA-99a is a microRNA that receives much attention; as a potential tumor marker, its biological functions are not identical in different tumor cells. For example, the expression of miRNA-99a is down-regulated in some tumors, such as head and neck squamous cell carcinoma[8], and oral cancer[9]. In some studies[10], it has also been confirmed that the overexpression of miRNA-99a has an inhibitory effect on the growth of hepatoma cells, and this inhibition is associated with the prognosis of patients with primary hepatocellular carcinoma. However, some studies[11] have shown that the expression of miRNA-99a is up-regulated in myeloid leukemia of children, and that miRNA-99a can facilitate the proliferation of leukemia cells and inhibit their apoptosis. Therefore, whether miRNA-99a is a carcinogenic factor or a tumor suppressor factor in tumors deserves to be further analyzed. In esophageal cancer, miRNA-99a can facilitate the proliferation, invasion and migration of cells, and inhibit the apoptosis of cells[12]. At present, the studies are few, which are about the changes of the expression levels of miRNA-99a and miRNA-224 in the progression of non-small cell lung cancer (NSCLC) and the correlation between them and the illness condition of patients with NSCLC. The expression of miRNA-99a and miRNA-224 in the serum of patients with NSCLC would be investigated, and the correlation between the expression changes of them and NSCLC would be analyzed this time.

Patients and Methods

Patients

83 patients with NSCLC, who were diagnosed and treated in our hospital from January 2014 to September 2017, were included in the experiment group, in which there were 45 males and 38 females, aged from 40 to 67 years; the average age of them was (53.64±11.63) years. At the same time, 79 patients, who made health check up in our hospital, were included in the control group, in which there were 36 males and 43 females, aged from 42 to 67 years, the average age of them was (53.45±13.95) years. Inclusion criteria: patients who were diagnosed as NSCLC by pathological diagnosis and received surgery were included in the experiment group; the healthy people, who were confirmed by physical examination, were included in the control group. Exclusion criteria: patients who had received chemoradiotherapy before the specimens were taken were excluded; patients with other severe organ diseases were excluded; patients who did not cooperate with the examination were excluded; the patients with cognitive disorder and communication disorder were excluded. All the patients agreed that their serum would be taken out during the operation to carry out the experiment, and they would cooperate with medical workers to complete the relevant diagnosis and treatment. The patients and their family were informed before the study was carried out and they signed the informed consent form. This study was approved by the Ethics Committee of the Shiyan Taihe Hospital. The differences in age, gender, obesity and smoking of the patients between the two groups were not statistically significant when their general data were compared ($p>0.05$), which were comparable (Table I).

Main Instruments and Reagents

TRIzol kit (ShenGong Biotechnology Co. Ltd, Shanghai, China); DNase1 (ShenGong Biotechnology Co. Ltd, Shanghai, China); cDNA reverse transcription kit (TaKaRa, Otsu, Shiga, Japan); ultraviolet spectrophotometer (Tongyong Technology Co., Ltd., Beijing, China); fluorescent quantitative Polymerase Chain Reaction kit (Bio-Rad, Hercules, CA, USA); Real Time-Polymerase Chain Reaction (RT-PCR) detector (ABI7 500) is a product of Applied Biosystems (Foster City, CA, USA).

Test Methods

The serum was taken out from a refrigerator in which the temperature was -80°C, and TRIzol reagent was added into it; RNA in the serum was extracted, then the ultraviolet spectrophotometer was used to test the purity of RNA. 1 μl of total RNA was taken and was operated according to instructions of the kits. Reverse transcription cDNA reaction parameters: 16°C, 15 min, 42°C, 60 min, 85°C, 5 min. The transcribed DNA was used for PCR amplification, and the amplification system of PCR was configured according to instructions of manufacturers. U6 was used as the internal reference

Table I. The general data of the patients [n (%)].

Group	The experiment group (no.=83)	The control group (no.=79)	X^2	*p*
Age (years old)				
<35	33 (39.76)	36 (45.57)	0.559	0.455
≥35	50 (60.24)	43 (54.43)		
Gender				
Male	40 (48.19)	39 (49.37)	0.022	0.881
Female	43 (51.81)	40 (50.63)		
Weight (kg)				
<55	36 (43.37)	32 (40.51)	0.137	0.712
≥55	47 (56.63)	47 (59.49)		
Have obesity or not				
Yes	44 (53.01)	42 (53.16)	0.001	0.985
No	39 (46.99)	37 (46.84)		
Whether smoking or not				
Yes	46 (55.42)	38 (48.10)	0.869	0.351
No	37 (44.58)	41 (51.90)		
Whether drinking or not				
Yes	33 (39.76)	30 (37.97)	0.054	0.816
No	50 (60.24)	49 (62.03)		
Pathological stage				
I-II stage	44 (53.01)	–	–	–
III-IV stage	39 (46.99)	–		

of PCR, and the primer sequences are shown in Table II. The PCR reaction conditions were: pre-degeneration at 95°C for 12 min, then 95°C for 15 s, 65°C for 30 s; 40 cycles were made, and the relative expression levels of genes were expressed in the form of $2^{-\Delta CT}$. ΔCT = ct in the serum of the experiment group - ct in the serum of the control group.

Statistical Analysis

SPSS 20.0 software package (IBM, Armonk, NY, USA) was used to statistically analyze the experimental data, and GraphPad Prism 7 software (La Jolla, CA, USA) was used to draw the experiment pictures; the measurement data were expressed in the form of mean value ± standard deviation; *t*-test or variance was used in the analysis between the two groups; chi-square test was used in the enumeration data. One-way ANOVA was used after Dunnett's *t*-test. When $p<0.05$, the differences were statistically significant.

Results

The Expressions of MiRNA-99a and MiRNA-224 in the Serum of the Patients in the Two Groups

The expression levels of miRNA-99a in the serum of the patients with NSCLC and healthy people were respectively (0.88±0.26) and (1.37±0.41); when the two groups were compared with each other, the expression level of miRNA-99a in the patients with NSCLC was remarkably lower than that in the patients in the normal control group, with statistically significant differences ($p<0.001$). The expression levels of miRNA-224 in the serum of the patients with NSCLC and healthy people were respectively (4.08±0.79) and (1.36±0.27); when the two groups were compared with each other, the expression level of miRNA-224 in the patients with NSCLC was markedly higher than that in the patients in the normal control group, with statistically significant differences ($p<0.001$; Table III).

Table II. The primers of miRNA-99a, miRNA-224 and U6.

Group	Forward primer	Reverse primer
miRNA-99a	5'-GATAACCCGTAGATCCGAT-3'	5'-GTGCGTGTCGTGGAGTCG-3'
miRNA-224	5'-GAGCCCAAGTCACTAGTGGT-3'	5'-GTGCAGGGTCCGAGGT-3'
U6	5'-ATTGGAACGATACAGAGAAGATT -3'	5'-GGAACGCTTCACGAATTTG -3'

Table III. The expressions of miRNA-99a and miRNA-224 in the serum of the patients in the two groups.

Group	The experiment group (no.=83)	The control group (no.=79)	*t*	*p*
The expression level of miRNA-99a	0.88±0.26	1.37±0.41	9.195	<0.001
The expression level of miRNA-224	4.08±0.79	1.36±0.27	28.96	<0.001

The Relationship Between the Expression Level of MiRNA-99a and the Clinicopathological Features of the Patients

The expression level of miRNA-99a in NSCLC was not significantly correlated with gender, age, body weight, pathological grade and diameter of tumor ($p>0.05$), and was markedly correlated with pathological stage, presence and absence of lymph node metastasis and tissue differentiation ($p<0.001$); the expression levels of miRNA-99a in the serum of the patients with NSCLC who had lymph node metastasis and in the serum of the patients with NSCLC who didn't have lymph node metastasis were respectively (0.69±0.25) and (1.07±0.31), and the expression level of miRNA-99a in the serum of the patients who didn't have lymph node metastasis was markedly higher than that of the patients who had lymph node metastasis, with statistically significant differences ($p<0.001$). The relative expression levels of miRNA-99a in I-II stage and III-IV stage of the pathological stage of NSCLC were respectively (0.61±0.21) and (1.15±0.34), and the expression level of miRNA-99a in I-II stage was remarkably lower than that in III-IV stage, with statistically significant differences ($p<0.001$). The relative expression levels of miRNA-99a in high differentiation, middle differentiation and low differentiation of pathological parts of the patients with NSCLC were respectively (1.05±0.26), (0.84±0.20) and (0.75±0.15), and the expression level of miRNA-99a in low differentiation was lower than that in middle differentiation, without statistically significant differences ($p>0.05$). The expression level of miRNA-99a in low differentiation was markedly lower than that in high differentiation, with statistically significant differences ($p<0.05$). The expression level of miRNA-99a in middle differentiation was remarkably lower than that in high differentiation, and the differences were statistically significant ($p<0.05$; Table IV).

Table IV. The relationship between the expression level of miRNA-99a and the clinicopathological features of the patients.

Group	No	miRNA-99a	*t*/F	*p*
Gender				
Male	40	0.82±0.22	1.11	0.270
Female	43	0.94±0.19		
Age (years old)				
<35	33	0.78±0.16	0.204	0.839
≥35	50	0.98±0.25		
Weight (kg)				
<55	36	0.81±0.27	1.556	0.123
≥55	47	0.91±0.31		
Pathological stage				
I-II stage	44	0.61±0.21	8.812	<0.001
III-IV stage	39	1.15±0.34		
Pathological grade				
G1+G2	37	0.84±0.30	1.822	0.072
G3	46	0.95±0.25		
Lymph node metastasis				
Yes	36	0.69±0.25	6.182	<0.001
No	47	1.07±0.31		
Diameter of tumor (cm)				
≤5	46	0.85±0.22	1.094	0.2774
>5	37	0.91±0.28		
Tissue differentiation				
High	25	1.05±0.26	18.59	<0.001
Middle	21	0.84±0.20		
Low	37	0.75±0.15		

The Relationship Between the Expression Level of MiRNA-224 and the Clinicopathological Features of the Patients

The expression level of miRNA-224 in NSCLC was not remarkably correlated with gender, age, body weight, tissue differentiation and diameter of tumor ($p>0.05$), and was significantly correlated with pathological stage, presence and absence of lymph node metastasis and pathological grade ($p<0.001$). The expression levels of miRNA-224 in the serum of the patients with NSCLC who had lymph node metastasis and in the serum of the patients with NSCLC who didn't have lymph node metastasis were respectively (5.21±0.46) and (2.95±0.27). The expression level of miRNA-224 in the serum of the patients who had lymph node metastasis was markedly higher than that of the patients who didn't have lymph node metastasis, with statistically significant differences ($p<0.001$). The relative expression levels of miRNA-224 in I-II stage and III-IV stage of the pathological stage of NSCLC were respectively (3.07±0.43) and (5.09±0.62), the expression level of miRNA-224 in stage I-II was significantly lower than that in stage III-IV, with statistically significant differences ($p<0.001$). The relative expression levels of miRNA-224 in G1+G2 and G3 of the pathological grade of the patients with NSCLC were respectively (3.18±0.31) and (4.98±0.42), and the expression level of miRNA-224 in G1+G2 was lower than that in G3, with statistically significant differences ($p<0.05$; Table V).

The Correlation Between the Expression of MiRNA-99a and the Expression of MiRNA-224 in NSCLC

The results of partial correlation analysis showed that the expression levels of miRNA-99a and miRNA-224 were negatively correlated with each other in NSCLC ($r=-0.985$, $p<0.001$; Figure 1).

Discussion

The division speed of NSCLC tumor tissue is slow, and the spread of which is late and the clinical symptoms are not significant, so it can't be found until the middle and advanced stage[13]. Scholars[14,15] have shown that the identification of early clinical stage and histological type is of great significance for the later treatment, and

Table V. The relationship between the expression level of miRNA-224 and the clinicopathological features of the patients.

Group	No.	miRNA-224	t/F	p
Gender				
Male	40	4.01±0.76	0.811	0.420
Female	43	4.15±0.81		
Age (years old)				
<35	33	4.12±0.69	0.675	0.502
≥35	50	4.23±0.75		
Weight (kg)				
<55	36	3.98±0.72	1.315	0.192
≥55	47	4.18±0.66		
Pathological stage				
I-II stage	44	3.07±0.43	17.40	<0.001
III-IV stage	39	5.09±0.62		
Pathological grade				
G1+G2	37	3.18±0.31	21.73	<0.001
G3	46	4.98±0.42		
Lymph node metastasis				
Yes	36	5.21±0.46	28.00	<0.001
No	47	2.95±0.27		
Diameter of tumor (cm)				
≤5	46	4.01±0.35	0.839	0.404
>5	37	3.94±0.41		
Tissue differentiation				
High	25	4.42±0.42	1.174	0.314
Middle	21	4.23±0.38		
Low	37	4.31±0.45		

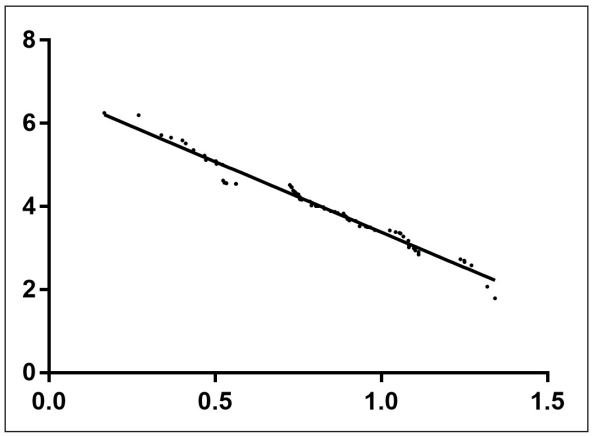

Figure 1. The correlation between the expression of miRNA-99a and the expression of miRNA-224 in NSCLC. The results of partial correlation analysis show that the expression levels of miRNA-99a and miRNA-224 are negatively correlated with each other in NSCLC (r=-0.985, p<0.001).

the traditional test method is mainly pathological biopsy, even though the accuracy is high but it is unbearable for patients who receive invasive manipulation. MiRNA-99a has different biological effects in different tumors. Wong et al[16] have reported that miRNA-99a is highly expressed in patients with epithelial cancer and tongue epithelium carcinoma, so it is speculated that miRNA-99a may be a proto-oncogene, but it is lowly expressed in lung cancer, suggesting that miRNA-99a may be a tumor suppressor gene[17]. Guo et al[18] demonstrated that miR-224-3p plays a role as a tumor suppressor gene in human glioblastoma cells, and that miR-224-3p not only can reduce the proliferation of cells, but also apparently facilitate apoptosis in hypoxia in U251 cell line and U87 cell line. Therefore, whether miRNA-99a and miRNA-224 can be used as tumor suppressor genes remains controversial. The studies about the expression and mechanism of miRNA-99a and miRNA-224 in NSCLC are a few. This work investigated the relationship between the expressions of miRNA-99a and miRNA-224 in the serum of the patients with NSCLC and the clinicopathological parameters, and analyzed the correlation between miRNA-99a and miRNA-224 in NSCLC.

First, qRT-PCR was used to investigate the expression levels of miRNA-99a and miRNA-224 in the serum of the patients with NSCLC, and the expression level of miRNA-99a in the patients with NSCLC was markedly lower than that in the patients in the normal control group, with statistically significant differences (p<0.001). The expression level of miRNA-224 in the patients with NSCLC was remarkably higher than that in the patients in the normal control group, and the differences were statistically significant (p<0.001). This indicates that miRNA-224 is highly expressed in NSCLC and miRNA-99a is lowly expressed in NSCLC. Zhang et al[19] found that mir-224 facilitates the proliferation of NSCLC cells by regulating the expression of RASSF-8. Chen et al[20] have shown that the expression of mir-99a is down-regulated in NSCLC, thus enhancing the IGF-1R pathway to regulate proliferation, migration and the formation of the colony, which is similar to the results of this paper. Next, the relationship between the expression levels of miRNA-99a and miRNA-224 and the clinicopathological features of the patients in the experiment group was analyzed, and the expression level of miRNA-99a in NSCLC was not markedly correlated with gender, age, body weight, pathological grade and diameter of tumor (p>0.05), but was significantly correlated with pathological stage, presence and absence of lymph node metastasis and tissue differentiation (p<0.001). The expression level of miRNA-224 in NSCLC was not remarkably correlated with gender, age, body weight, tissue differentiation and diameter of tumor (p>0.05), but was significantly correlated with pathological stage, presence and absence of lymph node metastasis and pathological grade (p<0.001). Currently, there are a few studies about the relationship and correlation between the expression levels of miRNA-99a and miRNA-224 and the clinicopathological features of patients with NSCLC. Yu et al[21] found that mir-99a targets the AKT1 signal pathway to inhibit the metastasis of NSCLC cells. Wang et al[22] analyzed mir-224 and the clinicopathological features of patients with NSCLC, who were tested by qRT-PCR technique, and found that mir-224 facilitates the proliferation of NSCLC cells by regulating the expression of RASSF-8, which can support the credibility of the results of this work. Finally, the correlation between the expression of miRNA-99a and the expression of miRNA-224 in NSCLC was analyzed, and the results showed that the expression levels of miRNA-99a and miRNA-224 were negatively correlated with each other in NSCLC (r=-0.985, p<0.001); currently there is no study about the correlation between miRNA-99a and miRNA-224 in NSCLC. In this work, NSCLC was used as a specific study object, and the expressions of miRNA-99a

and miRNA-224 and the clinicopathological features of NSCLC were specifically investigated. The paper's results have some significance for the clinical treatment of NSCLC. Finally, it is believed that monitoring the expression changes of miRNA-99a and miRNA-224 in the serum has some clinical therapeutic significance for the occurrence and development of NSCLC.

Conclusions

We found that miRNA-99a and miRNA-224 may be involved in the development and progression of NSCLC. MiRNA-99a is associated with NSCLC pathological stage, lymph node metastasis and tissue differentiation, while miRNA-224 is associated with NSCLC pathological stage, lymph node metastasis and pathological grade. MiRNA-99a and miRNA-224 have important value for the detection of NSCLC. However, this work also has some deficiencies. For example, the pathological mechanism of NSCLC was not investigated. Therefore, it is desirable that scholars could increase the sample size and further investigate the effects of miRNA-99a and miRNA-224 on NSCLC.

Conflict of Interests

The authors declare that they have no conflict of interest.

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