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SHORT COMMUNICATION

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miR-544a expression in NSCLC cell lines by real-time PCR

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ABSTRACT

Objective: To study miR-544a expression in NSCLC cell lines 95C and 95D. **Methods:** Cell culture by routine methods, total RNA extracted and reverse to cDNA. miRNA expression in 95C and 95D was analyzed by real-time PCR; over expression miRNA in 95D was transfected by liposomal to 95C and its invasion was detected by transwell test. **Results:** The expression of miR-544a was significantly increased in 95-D in QPCR tests ($P<0.05$); we transfected miR-544a mimic to 95C and observed that the invasion of 95C was significantly up regulation by transwell test ($P<0.05$). **Conclusions:** We successfully establish real-time PCR to examine miRNA expression and miR-544a may be related to the the invasion of NSCLC

KEYWORDS

miR-544a;
Real-time PCR;
NSCLC;
Invasion.

INTRODUCTION

miRNA has become a hot area of research in recent years. Many studies have shown that miRNA participates in a series of life activities. miRNA mediates the Occurrence, development of tumors. So miRNA research can contribute to tumor study. Mature miRNA usually consists of 21-23 bases. miRNA can combine with 3' UTR of target genes through the second to eighth bases of the 5 'end. So target genes may degrade or their translation may be inhibited, resulting in their down regulation[1].

Non-small cell lung cancer (NSCLC) occupies 80% of all lung cancer[2] and it is necessary to study NSCLC. In NSCLC cell lines, as to 95C, the metastasis of 95D is superior. Preliminary miRNA array experiments show that miR-544a expression increases significantly in 95D. In this study, real-time PCR method is used to study the expression of miR-544a and prove the results of miRNA array. miR-544a is transfected to 95C and its invasion and metastasis ability is detected by transwell tests. Through the above tests, the ability of miR-544a in the metastasis of NSCLC is studied.

MATERIALS AND METHODS

Real-time PCR

snRNA U6 is served as internal control.

The primer of miR-544a is from Rui Bo Company. miRNA expression of different groups is compared by the value of $2^{-\Delta\Delta CT}$.

(a) Total RNA extraction

1ml of Trizol Reagent (Invitrogen) was added to cellines and the suspension was homogenized. 0. 2 ml of chloroform/1ml of TRIZOL was added and shaken by hand for 15 seconds and maintained for 2 minutes at 15°C -30°C. The samples were centrifuged at 12000g for 15 minutes at 4 °C: the aqueous phase that contained RNA was removed and transferred to a new tube with the sanme volume isopropanol. After 30 minutes at -20 °C, samples were centrifuged at 12000g for 10 minutes at 4 °C. The supernatant was removed and the RNA pellet washed with 1.0 ml of 75% ethanol. After re-suspension of the pellet, the tube was centrifuged at 7500g for 10 minutes at 4 °C, the supernatant removed and the RNA pellet dried in air for 5 minutes. The RNA re-suspended in RNase-free-water (20-50µl, depending on the amount of the RNA pellet) and incubated at 55 °C for 5 minutes, then stored at -80 °C.

(b) Reverse transcriptase

The reverse transcriptase reaction was performed according the protocol of the SuperScript III First-Strand Synthesis SuperMix (Invitrogen) by using 2 µg RNA previously treated with DNase 1, a reverse transcriptase enzyme (Invitrogen), random hexamers as primers, dNTPs and an RNase inhibitor. The resulting cDNA can be directly used for the Real Time PCR or stored at -20 °C.

(c) Real-Time PCR

Quantitative PCR was performed using PCR Detection System (Bio-Rad) with the use of SYBR Green I Premix Ex Taq (TaKara). Specific primers for miR-544a were designed by Rui Bo Company. The reaction protocol included an initial step of 120 sec at 95 °C. Each PCR cycle involved denaturation (95 °C, 30 sec), annealing (60 °C, 35sec) and extension (72 °C, 20 sec). After 40 cycles, another step was set: 95°C 15s, 60°C 30s, 95°C 15s. Fluorescence was measured at each cycle. The relative fold change of expression of miR-544a was quantified as $2^{-\Delta\Delta CT}$, where ΔΔCt was Ct (target genes) - Ct (housekeeping gene). We selected snRNA U6 as housekeeping gene. Its primer is as followed:

upper stream primer : TGGCACCCAGCACAATGAA;

downstream primer : CTAAGTCATAGTCCGCCTAGAAGCA.

Cell transfection and cell migration analysis

Cells were transfected with the indicated vector(s) using the Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's protocol, that's, cell density was 30% and the concentration of miRNA was 100nmol/L. We transfected 95C with miR-544a mimic and 95D with miR-544a inhibitor respectively. miR-544a inhibitor was used as a negative control. After transfection, cells were incubated in complete medium at 37° C in 5% CO_2 for 48h and the NSCLC cell migration analysis was carried out using Transwell inserts. Cells (5×104) in 250 µl of serum-free medium RPMI 1640 were seeded onto filters in 24-well plates. The medium (550 µl) containing RPMI 1640 supplemented with 101% fetal bovine serum was placed in the lower chamber. After incubation for 24 h, non-invading cells on the top of each Transwell were scraped off with a cotton swab. Cells that had migrated to the other side were fixed with methyl alcohol and glacial acetic acid (3:1) and stained with hematoxylin. The number of migrated cells was manually counted with a light microscope under 200 × magnifications. The sum of the numbers of cells in five areas was used as the migrated cell number. These experiments were repeated at least three times, and significant differences were assessed by SPSS 15.0 followed by T test.

RESULTS

miR-544a expression by real-time PCR

We perform real-time PCR to validate miR-544a expression in NSCLC cell lines. The results showed that miR-544a expression in NSCLC 95D (high cell migration) was significantly unregulated ($P<0.05$). The results were shown in Figure 1.

Cell migration analyses by Transwell inserts

We transfected 95C with miR-544a mimic and 95D with miR-544a inhibitor respectively. And then cell migration analyses were done by Transwell inserts. Cell migration ability of transfected 95C was significantly higher ($P<0.05$) and that of transfected 95D was significantly lower ($P<0.05$), seen in figure 2.

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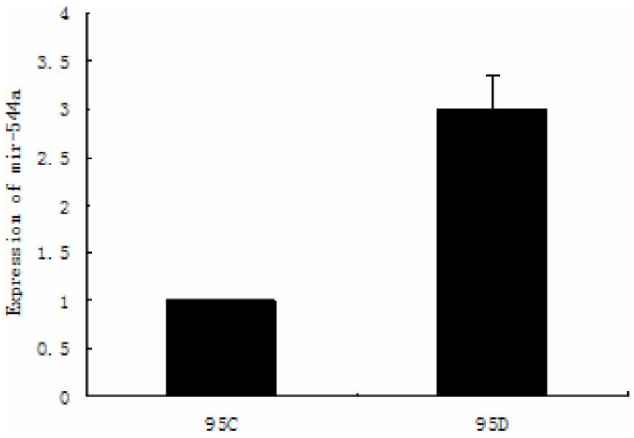

Figure 1 : Real-time PCR showed that miR-544a expression in NSCLC 95D was higher (P<0.05)

DISCUSSION

Many studies revealed that miRNA is related to tumor migration and invasion, but its mechanism is not clear. In our early studies, we found that miR-544a expression in NSCLC 95D was higher by miRNA array. However, the results of miRNA array may be false positive and the results should be validated by other tests. Real-time PCR is so sensitive to be used to detect miR-544a expression. The aim of this study is to establish the method of real-time PCR to validate the results of miRNA array. We extract total RNA and reverse RNA to cDNA. snRNA U6 was selected as housekeeping gene. Expression of miR-544a was quantified as $2^{-\Delta\Delta CT}$. The results of real-time PCR also revealed that miR-544a expression in NSCLC 95D was higher (P<0.05). This method successfully validated the results of miRNA array.

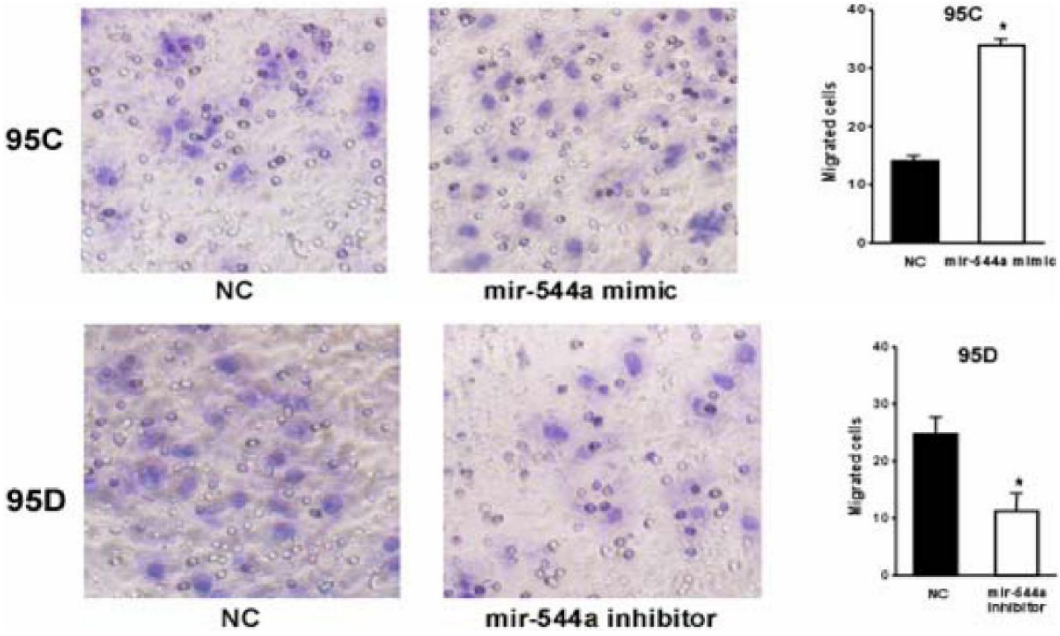

Figure 2 : Transwell inserts showed that cell migration ability of transfected 95C was significantly higher (P<0.05)

In order to study the effect of miR-544a in NSCLC, we transfected 95C with miR-544a mimic and 95D with miR-544a inhibitor respectively. Transwell inserts were used to detect cell migration. Cell migration ability of transfected 95C was significantly higher (P<0.05). This revealed that miR-544a may be related to the migration an invasion of NSCLC. Other miRNA studies also found similar results in other tumor[3,4]. We will do more studies to explore the mechanism of miR-544a in NSCLC. And this may provide us more ways to diagnose and treat NSCLC.

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