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The expressions of miR-151a-5p and miR-23b in lung cancer tissues and their effects on the biological functions of lung cancer A549 cells

S. GUO, J. ZHANG, Y.-Y. ZHAO, L.-Y. ZHOU, Y. XIE, X.-Y. WU, X. BIAN, X.-Y. YU

Department of Respiratory Medicine, Shandong Provincial Chest Hospital, Jinan, P.R. China

Abstract. – OBJECTIVE: To investigate the expressions of miR-151a-5p and miR-23b in lung cancer tissues and their effects on the biological functions of lung cancer A549 cells.

PATIENTS AND METHODS: Samples of lung cancer tissue (55 cases) and pericarcinomatous tissue (55 cases) were collected in thoracic surgery in our hospital from May 2017 to November 2018. The expression levels of miR-151a-5p and miR-23b in lung cancer tissues and pericarcinomatous tissues were detected by RT-PCR. Lung cancer cells A549 were transfected. Before transfection, the cells were divided into a negative control group (NC group, transfected with miRNA NC), a group transfected with miR-151a-5p inhibitor and a group transfected with miR-23b inhibitor. MTS Cell Proliferation Colorimetric Assay Kit (CCK8) was used to detect cell proliferation and draw the growth curve. Transwell chamber was used to detect the invasion ability *in vitro*, and BD flow cytometry was used to detect apoptosis in each group.

RESULTS: The expression levels of miR-151a-5p and miR-23b in lung cancer tissues were significantly higher than those in pericarcinomatous tissues ($p<0.001$). After 48 h to 72 h, the cell growth of both the miR-151a-5p inhibitor group and the miR-23b inhibitor group was significantly lower than that of the NC group ($p<0.001$). The numbers of invasion of miR-151a-5p inhibitor group and miR-23b inhibitor group were significantly lower than that of NC group ($p<0.00$). The apoptosis rates of miR-151a-5p inhibitor group and miR-23b inhibitor group were significantly higher than that of NC group ($p<0.001$).

CONCLUSIONS: Both miR-151a-5p and miR-23b are highly expressed in lung cancer, and the inhibition of miR-151a-5p and miR-23b can restrain the proliferation, invasion and migration of lung cancer A549 cells, thereby promoting the apoptosis of lung cancer A549 cells.

Key Words:
MiR-151a-5p, MiR-23b, A549, Cell biological function, Lung cancer.

Introduction

Lung cancer is a highly invasive and heterogeneous disease. It causes more death than breast cancer, prostate cancer and colon cancer each year[1-3]. Although the development of clinical radiochemical treatment for lung cancer grows rapidly, the long-term survival rate of patients is still very low[4]. The cause of lung cancer is not clear at present, but relevant results show that long-term exposure to air pollution of combustion related fine particle may be an important cause of lung cancer[5]. Patients have a low survival rate after treatment. Therefore, clinical research of new therapeutic direction has become a hot spot.

MicroRNA (mirRNA) is a small non-coding RNA that regulates gene expression through translation inhibition and mRNA incision[6]. Bioinformatics shows that 60% of protein-coding genes can be regulated by miRNA and are involved in a variety of biological processes, which can promote the development of various types of cancer[7]. miR-151a-5p expresses abnormally in thyroid papillary carcinoma, breast cancer, prostate cancer and other tumors[8,9]. It has been reported that miR-151a plays a role in non-small cell lung cancer by targeting epithelial cadherin genes and inducing proliferation, migration, and partial epithelial metastasis[10]. Abnormal expression of miR-23b was also found in ovarian cancer, gastric cancer, bladder cancer and other cancers[11-13]. MiR-23b could be considered as a new non-invasive biomarker for lung cancer patients[14]. Therefore, miR-151a-5p and miR-23 can play a role as tumor suppressor or oncogene. Clinically, there are few studies in the abnormal expressions of miR-151a-5p and miR-23b in lung cancer. In this study, the expression of miR-151a-5p and miR-23b in lung cancer and their influence on the biological function of lung cancer cells A549 were investigated.

Corresponding Author: Xueyan Yu, MM; e-mail: yxydqy@163.com

Patients and Methods

Collection of Tissue Samples

Samples of lung cancer tissue (55 cases) and pericarcinomatous tissue (55 cases) were collected in thoracic surgery in our hospital from May 2017 to November 2018.

Exclusion and Inclusion Criteria

Inclusion criteria: patients underwent imaging examination and confirmed to have a single liver tumor or up to 3 nodules (each nodule≤3 cm)[15], and patients with normal mental status. All subjects and their families have been informed and signed the informed consent in this study. This investigation has been approved by the Ethics Committee of our hospital.

Exclusion criteria: patients received any previous chemoradiotherapy; patients with severe liver and kidney dysfunction; patients with blood diseases; pregnant or lactating women.

Main Reagents and Instruments

Human lung cancer cell line A549 (Shanghai Guandao Biological Engineering Co., Ltd.), real-time fluorescence quantitative PCR instrument (Shanghai Shiwei Experimental Instrument Technology Co., Ltd.), cell apoptosis kit (Sino Biological Inc.), cell cycle detection kit (Beijing Baiaolaibo Technology Co., Ltd.), 10% fetal calf serum (FCS; Shanghai Xibao Biotechnology Co., Ltd.), TRIzol reagent (Henan Jiaozuo Lufeifan Biotechnology Co., Ltd.), Dulbecco's Modified Eagle's Medium (DMEM) culture medium (Qingdao Jieshikang Biotechnology co., LTD.), transfection reagent Lipofectamine™ 2000 (US Everbright Inc.), CCK8 kit (Beijing g-clone biotechnology Co., Ltd.), transwell chamber (Beijing Dakewe Biotechnology Co., Ltd.), CyFlow Cube 8 flow cytometer (Beijing Emeitongde Technology Development Co., Ltd.), primer sequences and transfection plasmid synthesis design of miR-151a-5p, miR-23b and internal reference U6 (Shanghai Hepeng Biotechnology Co., Ltd.). More details were shown in Table I.

Detection Methods

Detection of miR-151a-5p and miR-23b

The expressions of miR-151a-5p and miR-23b in lung cancer tissues and pericarcinomatous tissues were detected by PCR, and total RNA was extracted from cells by TRIzol in strict accordance with operating instructions. The concentration and purity of total RNA were detected using UV spectrophotometer, and RNA with OD260/OD280 ratio between 1.8 and 2.0 was taken. cDNA was synthesized by reverse transcriptase and oligonucleotide according to the operating instructions. Transcription reaction system (20 μL): buffer 4 μL, reverse transcriptase 2 μL, total RNA 2 μL, enzyme water without RNA 12 μL. Reaction conditions: 42°C water bath for 1 hour, 95°C water bath for 5 minutes. PCR instrument was used for amplified reaction, and RNU6B was used as the internal reference. Specific primers of miR-151a-5p and miR-23b were used to detect the expressions of miR-151a-5p and miR-23b in the fluorescence quantitative PCR instrument according to the operating instructions (Table I). PCR reaction system (20 μL): upstream primers 0.4 μL, downstream primers 0.4 μL, miR 0.5 μL, the rest of the reaction system was supplemented with ddH2O. Real-time PCR conditions: 94°C for 10 seconds, 94 °C for 5 seconds, 52°C for 30 seconds annealing, 72°C for 15 seconds, and then 40 cycles. Three compound wells were set for each experiment, and the experiment was repeated three times. The experimental results were analyzed by relative quantitative method, and the expressions of miR-151a-5p and miR-23b were expressed as 2-ΔΔCT.

Culture and Transfection of Cells

High-glucose Dulbecco's Modified Eagle's Medium (DMEM) medium containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin was used for conventional serial subcultivation. The growth conditions should be in a cell incubator at 37°C and 5% CO_2, and the cells should be plated into 6-well plates. Before transfection, the non-transfected cells were divi-

Table I. Primer sequences of miR-151a-5p, miR-23b and internal reference U6.

	Sense primer	Reverse primer
miR-151a-5p	5'-ACGCGTCGAGGAGCTCACAG-3'	5'-ATCCAGTGCAGGGTCCGAGG-3'
miR-23b	5'-CSCGGCCGCTAGTATTATGTT-3'	5'-CACATTTTA AAAAACATA-3'
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-AACGCTTCACGAATTTGCGT-3'

ded into miR-151a-5p inhibitor group, miR-23b inhibitor group, negative RNA control group (NC group, transfected with miRNA NC). Lipofectamine TM2000 kit was used to transfect the cells according to the operation instructions, and the cells were transferred into the miR-151a-5p inhibitor group, miR-23b inhibitor group and NC group. Then, the expressions of miR-151a-5p and miR-23b transfected with in cell A549 were detected.

Detection of Cell Growth Curve

A549 cell lines after transfection of the four groups were inoculated in 96-well plates, and three complex wells. 2 hours before the end of training, 20 μL Cell Proliferation Colorimetric determination reagent (MTS Cell effort Colorimetric Assay Kit) was added to each well at three time points (24 h, 48 h, 72 h). After that, it was put in 5% CO_2 incubator at 37°C. Two hours later, automatic microplate reader at 490 nm wavelength was used to detect absorbance and observe cells proliferation. The experiment repeated 3 times.

Detection of Invasion Ability of Cells In Vitro

The cells were digested with trypsin and then re-suspended in serum-free medium. Resuspending (200 μL) containing about 5×104 cells was taken and 1 m medium containing FBS was added to 6-well subplate chamber. Cell suspension (2 ml) was added to transwell chamber. After 24 hours of conventional culture, the cells in transwells chamber were wiped off with a cotton swab. When the transwell chamber was dried, the membrane was prepared and sealed. The transwell cells were observed and counted under an optical microscope.

Detection of Apoptosis

Apoptosis was detected by a cell apoptosis detection box in accordance with the instructions. Cells were transfected for 48 h and dyed with AnnexinV, propidium iodide (PI) in 6-well plates were detected by BD flow cytometry (Franklin Lakes, NJ, USA). The experiments were repeated 3 times.

Targeting Relationship Between miR-151a-5p, miR-23b and IL1RAPL1 Detected By Dual Luciferase Reporter Gene

A DNA fragment of miR-151a-5pmRNA and miR-23bmRNA 3'-UTR containing the putative binding site of IL1RAPL1 was synthesized. The fragment was then subcloned into the Xho I and Not I sites downstream of the *Renilla* Luciferase coding region of the psiCHECK-2 vector and verified by Sangon Biotech sequencing. PsiCHECK2-IL1RAPL1-wt or PsiCHECK2-IL1RAPL1-mut was constructed, and miR-151a-5pinhibitor and miR-23binhibitor or miR-NC and Wt-IL1RAPL1 or Mut-IL1RAPL1 were transfected into A549 cells using LipofectamineTM2000. Cells were harvested 48 hours after transfection and analyzed for firefly and *Renilla* Luciferase activity using the Dual-Luciferase reporter assay (Promega, Madison, WI, USA) according to the manufacturer's instructions.

Statistical Analysis

Differences were verified by SPSS 20.0 (IBM, Armonk, NY, USA) statistical software. The *t*-test was used to analyze measurement data, which was expressed as mean ± standard deviation ($\bar{x} \pm sd$). Analysis of variance (ANOVA) *F* was used for test among the three groups. Repeated Measures ANOVA analysis was used for comparison of multiple time points in groups. $p<0.05$ was considered as statistically significant difference.

Results

Expressions of MiR-151a-5p and MiR-23b In Lung Cancer Tissues and Pericarcinomatous Tissues

The relative expression levels of miR-151a-5p in lung cancer tissues and pericarcinomatous tissues were (5.72±0.42) and (1.21±0.03), respectively. The relative expression levels of miR-23b in lung cancer tissues and pericarcinomatous tissues were (6.33±0.39) and (2.01±1.05), respectively. The relative expression level of miR-151a-5p in lung cancer tissues was significantly higher than that in pericarcinomatous tissues, with statistically significant differences ($p<0.001$). The relative expression level of miR-23b in lung cancer tissues was also significantly higher than that in pericarcinomatous tissues, with statistically significant differences ($p<0.001$; Figure 1).

Expression Levels of MiR-151a-5p and MiR-23b In Each Group after Transfection

The relative expression of miR-151a-5p in the miR-151a-5p inhibitor group after transfection

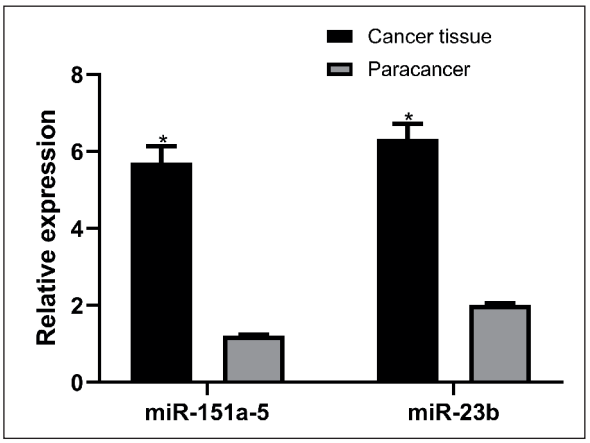

Figure 1. Expressions of miR-151a-5p and miR-23b in lung cancer tissues and pericarcinomatous tissues. The expression level of miR-151a-5p in lung cancer tissues was significantly higher than that in pericarcinomatous tissues, with statistically significant differences (p<0.001). The expression level of miR-23b in lung cancer tissues was also significantly higher than that in pericarcinomatous tissues, with statistically significant differences (p<0.001). Note: *Means the expression level of miR-151a-5p or miR-23b compared with cancer tissues (p<0.05).

of the inhibitor was (0.58 ± 0.12); the relative expression of miR-23b in the miR-23b inhibitor group was (0.62 ± 0.14); the relative expression of the NC group was (1.13 ± 0.22). The results showed that the relative expression of miR-151a-5p inhibitor group and miR-23b inhibitor group was significantly lower than that of NC group (p <0.05; Figure 2).

Comparison of Cell Growth of Human Lung Cancer Cell Line A549 at Different Time Periods

At 24 h, there was no significant difference in A549 cell growth between the groups (p> 0.05); from 48 h to 72 h, the growth of A549 cells in miR-151a-5p inhibitor group and miR-23b inhibitor group was significantly lower than that in NC group (p <0.05; Figure 3).

Comparison of the Invasion of Cell Line A549 In Each Group

The invasion numbers of miR-151a-5p inhibitor group, miR-23b inhibitor group, NC group were (85.37±8.12), (86.21±7.83), (168.48±12.42), respectively. Numbers of invasion in the miR-151a-5p inhibitor group and miR-23b inhibitor group were significantly lower than those in NC group. The differences were statistically significant (p<0.001; Figure 4).

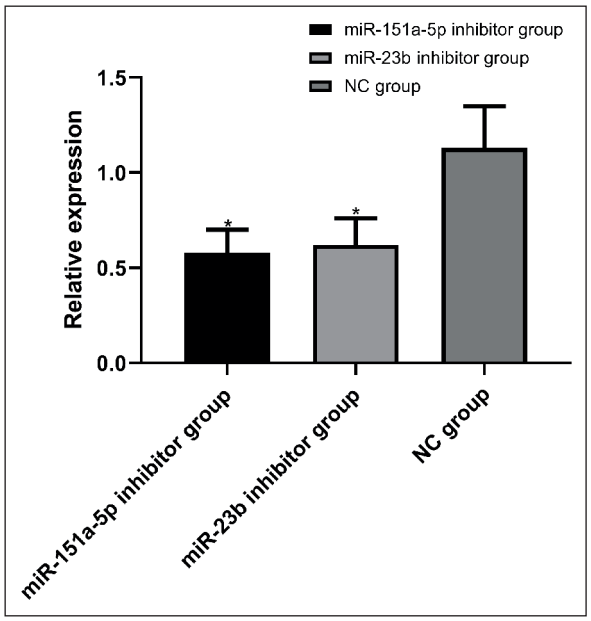

Figure 2. MiR-151a-5p and miR-23b expression in cells of each group after transfection. The relative expression of miR-151a-5p inhibitor group and miR-23b inhibitor group was significantly lower than that of NC group (p<0.05). Note: *Indicates comparison with NC group (p<0.05).

Comparison of Apoptosis Ability of Human Lung Cancer Cell Line A549 After Transfection In Each Group

The apoptosis rates of miR-151a-5p inhibitor group, miR-23b inhibitor group, NC group we-

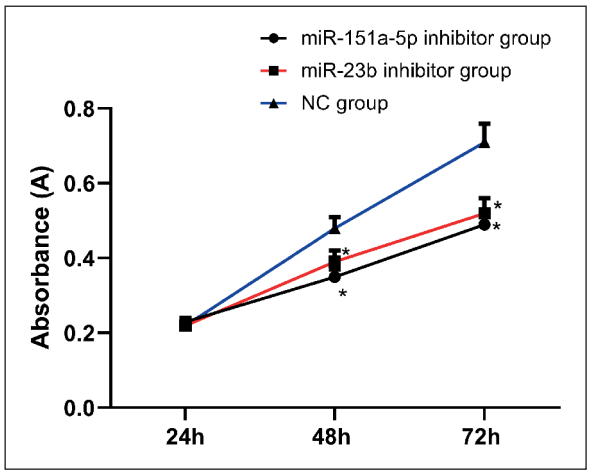

Figure 3. A549 cell growth in different groups at different time periods. At 24 h, there was no significant difference in A549 cell growth between the groups (p> 0.05); from 48 h to 72 h, the growth of A549 cells in miR-151a-5p inhibitor group and miR-23b inhibitor group was significantly lower than that in NC group (p<0.05). Note: *Indicates comparison with the NC group (p<0.05).

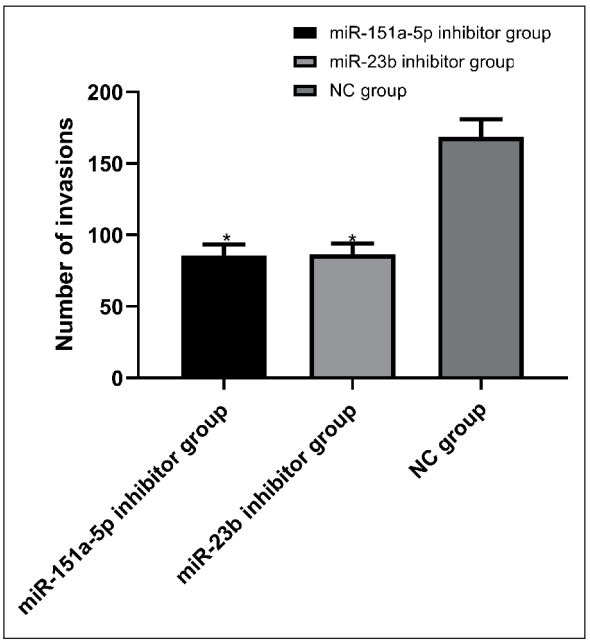

Figure 4. A549 cell invasion. A549 cell invasion in the miR-151a-5p inhibitor group and miR-23b inhibitor group was significantly lower than that in the NC group, and the differences were statistically significant (p<0.001). Note: *Indicates a comparison with the NC group (p<0.05).

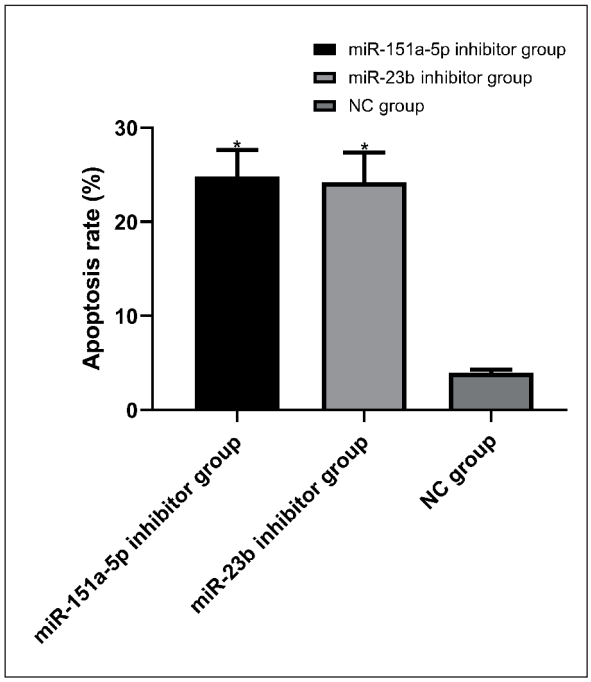

Figure 5. A549 cell apoptosis. The apoptosis rate of miR-151a-5p inhibitor group and miR-23b inhibitor group was significantly higher than that of NC group, and the differences were statistically significant (p<0.001). Note: *Indicates comparison with NC group (p<0.05).

re (24.79±2.84)%, (24.19±3.17)%, (3.94±0.38)%, respectively. The apoptosis rates in the miR-151a-5p inhibitor group and miR-23b inhibitor group were significantly higher than those in NC group, the differences were statistically significant (p<0.001; Figure 5).

Binding Sites Between MiR-151a-5p, MiR-23b and IL1RAPL1

It was predicted that there were binding sites between miR-151a-5p, miR-23b and IL1RAPL1. The Dual-Luciferase reporter gene results showed that miR-151a-5pinhibitor and miR-23b inhibitor can reduce the Luciferase activity of IL-1RAPL1-Wt, but it does not affect the Luciferase activity of IL1RAPL1-Mut, indicating that miR-151a-5p and miR-23b combined with IL1RAPL1 significantly reduced fluorescence activity (p <0.05; Figure 6).

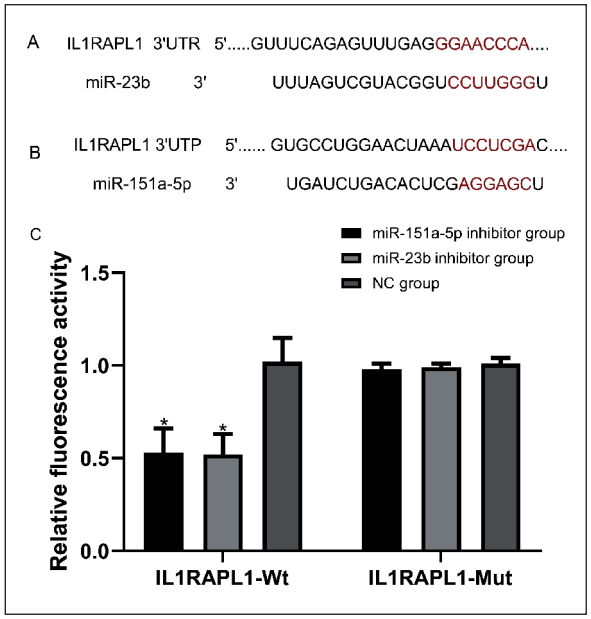

Figure 6. Results of the Dual-Luciferase reporter gene. MiR-151a-5pinhibitor and miR-23b inhibitor can reduce the Luciferase activity of IL1RAPL1-Wt, but it does not affect the Luciferase activity of IL1RAPL1-Mut, indicating that when miR-151a-5p and miR-23b fluoresce combined with IL1RAPL1, the activity was significantly reduced (p<0.05). Note: *Iindicates a comparison with the NC group (p<0.05).

Discussion

Lung cancer causes the largest number of new cases and cancer deaths each year worldwide[16]. Many patients were found to have advanced lung cancer. The cells of advanced lung cancer grew

and metastasized rapidly, and the radiochemical treatment had only a slight effect[17]. To increase the number of lung cancer survivors, new direction of this disease may be brought by the introduction of angiogenesis, epidermal growth factor receptor (EGFR) inhibitors and other new anticancer agent[18].

Targeted therapy is the therapeutic prospect to achieving more effective and rational cancer treatment by targeting molecules and approaches of abnormal kinetic energy[19]. More rational approaches are being developed to inhibit tumor growth, survival and spread. As a small group of non-coding RNA, miRNA plays an important role in many important biological processes[20], and is reported to regulate the expression of various oncogenes or tumor suppressor genes[21]. In this paper, miR-151a-5p and miR-23b, as members of the miRNA family, their expressions in lung cancer and effects of proliferation, migration and apoptosis on lung cancer cell A549 were studied.

In this work, PCR was used to detect the expressions of miR-151a-5p and miR-23b in lung cancer tissues and pericarcinomatous tissues. The results showed that the expressions of miR-151a-5p and miR-23b in lung cancer tissues were significantly higher than those in pericarcinomatous tissues, indicating that the abnormal increase of miR-151a-5p and miR-23b may cause the occurrence of cancer. In the study of Liang et al[22], mir-151a-5p was down-regulated in gemcitabine-resistant lung cancer cell lines, which showed that the miR-151a-5p was highly expressed in lung cancer cell line A549, which was similar to the results of this study. According Zhu et al[23], the expression level of miR-23b in untreated lung cancer patients was significantly higher than that in benign lung cancer patients and healthy controls, with statistically significant differences. Moreover, they believed that miR-23b could be considered as a new non-invasive biomarker for the diagnosis of lung cancer, which was similar to the results of this study. We also studied the expression levels of miR-151a-5p and miR-23b in cells of each group after transfection, the results showed that the expression level of miR-151a-5p in the transfected inhibitor group was significantly lower than those in the negative control group (NC group), and the expression level of mir-23b in the inhibitor group was also significantly lower than that in the NC group. This indicates that the expression of mir-151a-5p and mir-23b can be down-regulated in the treatment of lung cancer to reduce the proliferation of cancer cells. The growth and metastasis of cancer cells are manifestations of cancer deterioration and an important cause of death of patients. In this study, we observed and compared the cell growth of human cell line A549 at the time bucket of 24 h, 48 h and 72 h. The results indicated that there were no significant differences between the miR-151a-5p inhibitor group, miR-23b inhibitor group, NC group at 24 h. However, from 48 h to 72 h, the cell growth of miR-151a-5p inhibitor group and miR-23b inhibitor group was significantly lower than that of NC group, and the differences were statistically significant. These can also suggest that the suppression of miR-151a-5p and miR-23b can inhibit the growth of cancer cells. Kalluri and Weinberg[24] have shown that epithelial-mesenchymal transformation can induce migration of cancer cells and invasion of lung cancer cells. In the study of Daugaard et al[25], miR-151a plays an up-regulating role in the pathogenesis of lung cancer by regulating key gene products including e-cadherin, fibronectin and Slug, promoting tumor cell growth and inducing part of epithelial-mesenchymal transformation. Therefore, when the expression of miR-151a-5p and miR-23b is reduced, it may achieve tumor inhibition by inhibiting epithelial-mesenchymal transformation. The results of cell invasion showed that the number of cell invasion in miR-151a-5p inhibitor group and miR-23b inhibitor group was significantly lower than that in NC group, and the differences were statistically significant. But the apoptosis rates of miR-151a-5p inhibitor group and miR-23b inhibitor group were higher than that of NC group, with statistically significant differences. In the process of cancer treatment, if the growth of cancer cells is significantly inhibited and the apoptosis rate of cancer cells is significantly increased, it can be a sign that the deterioration of the patient's condition is inhibited. It can also indicate the improvement of the patient's condition, which is of great significance for the prognosis of patients. We found that miR-151a-5p and miR-23b may be jointly targeted to promote the development of lung cancer cells, but there is currently no related research in clinical practice.

Conclusions

To sum up, miR-151a-5p and miR-23b are highly expressed in lung cancer. Reduction of expressions of miR-151a-5p and miR-23b can inhibit the growth and migration of human cell line A549 and promote cell apoptosis. Therefore, miR-151a-5p and miR-23b can be potential targets

for the treatment of lung cancer, so as to provide clinical basis for clinical treatment. Moreover, we also found that miR-151a-5p and miR-23b have binding sites with IL1RAPL1, suggesting that miR-151a-5p and miR-23b may promote apoptosis by targeting IL1RAPL1.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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