

Original Article

MiRNA-153 regulates cell viability and the cell cycle in bladder cancer

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Abstract: Bladder cancer is the most common malignancy in the urogenital system. MiRNA-153 has become a biomarker for the clinical diagnosis and prognosis of multiple malignant tumors. This study analyzed the influence of miRNA-153 on bladder cancer cell viability and the cell cycle. Bladder cancer cell line T24 was cultured in vitro and transfected by miRNA-153 mimics, an miRNA-153 inhibitor, and a mimic control or an inhibitor control. Cell viability was assessed using an MTT assay. The cell cycle was determined using flow cytometry. Bcl-2, Mcl-1, and FOXO1 protein expressions were detected by Western blot. MiRNA-153 expression increased in the miRNA-153 mimic group, but miRNA-153 was reduced in the miRNA-153 inhibitor group compared to the mimic control, inhibitor control, and normal control groups ($P < 0.05$). Cell viability was alleviated in the miRNA-153 mimic group, but cell viability was enhanced in the miRNA-153 inhibitor group compared with the control ($P < 0.05$). Cell proportion in the S phase increased in the miRNA-153 mimic group, but it was attenuated in the miRNA-153 inhibitor group compared with the control ($P < 0.05$). The Bcl-2, Mcl-1, and FOXO1 proteins were downregulated in the miRNA-153 mimic group, but they were elevated in the miRNA-153 inhibitor group compared with the control ($P < 0.05$). In conclusion, miRNA-153 suppressed cell viability and arrested the cell cycle in bladder cancer T24 cells by inhibiting the Bcl-2, Mcl-1, and FOXO1 proteins.

Keywords: miRNA-153, bladder cancer, viability, cell cycle

Introduction

As one of the three major urinary tumors, bladder cancer is common in the clinic. It accounts for one out of seven malignant tumors worldwide. Bladder transitional cell carcinoma is the most common type of bladder cancer and accounts for more than 90% of cases. Tumor invasion, relapse, and metastasis are the leading causes affecting prognosis and death. Therefore, the treatment of bladder cancer has a long way to go [1]. Following the development of diagnosis and treatment technologies, more and more bladder cancers can be diagnosed and treated. In recent years, the limitations of bladder cancer diagnosis seriously blocked the improvement of treatment efficacy. MiRNAs provide a new means for the early diagnosis, prognosis determination, and treatment of cancer [2]. MiRNAs are a type of endogenous small molecule single-strand RNAs with a length of about 22 nt. They are widely expressed in dif-

ferent tissues and can affect biological behavior by blocking target gene transcription or inhibiting expression at the posttranscriptional level [3, 4]. Recent studies found that miRNAs play a crucial role in cancer, embryonic development, and metabolic diseases. MiRNA-153 (miR-153) is a target gene in various cancer cells that regulates cell viability, apoptosis, and invasion [5-7]. However, the role of miR-153 in bladder cancer occurrence and development has not been confirmed. This study transfected miR-153 to the bladder cancer cell line T24, and tested cell viability, the cell cycle, Bcl-2, Mcl-1, and FOXO1 protein expressions, aiming to analyze the influence of miR-153 on the T24 cell viability and cycle.

Materials and methods

Materials

Experimental cells: Bladder cancer cell line T24 was purchased from Bioleaf (Shanghai, China).

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Table 1. Primer sequences

Gene	Sequence (5'-3')
miR153 mimics	Forward 5'-AGCCGTCAAGAGCAATAACGAA-3'
	Reverse 5'-GTGCAGGGTCCGAGGT-3'
miR-153 inhibitor	Forward 5'-CTCAACTGTCGTGGAGTCGGCAATTCAGTTGAGGAACAGGT-3'
	Reverse 5'-GAGTTGACAGCACCTCAGCCGTTAAGTCAACTCCTTGCCA-3'
Mimic control	Forward 5'-ACACTCCAGCTGGGCCAGTGTTCAGACTAC-3'
	Reverse 5'-TGTGAGGTGACCCGGGTCAAGTCTGATG-3'
Inhibitor control	Forward 5'-TGGTGTCTGGAGTCG-3'
	Reverse 5'-ACCACAGCACCTCAGC-3'
U6	Forward 5'-CTCGCTTCGGCAGCACA-3'
	Reverse 5'-AACGCTTCACGAATTTGCGT-3'

secondary antibody were purchased from ZSbio (Beijing, China). RPMI-1640 medium and MTT were bought from Sigma (USA). Flow cytometry was provided by Beckman (Germany). A cell incubator and an inverted microscope were obtained from SANYO (Japan).

Table 2. MiR-153 expression in T24 cells

Group	miRNA-153
miRNA-153 mimic	1.894 ± 0.075 ^{*,#,&}
miRNA-153 inhibitor	0.022 ± 0.003 ^{*,#,&}
mimic control	0.684 ± 0.012
Inhibitor control	0.667 ± 0.013
Normal control	0.712 ± 0.012

*P < 0.05: compared with mimic control, #P < 0.05: compared with inhibitor control, &P < 0.05: compared with normal control.

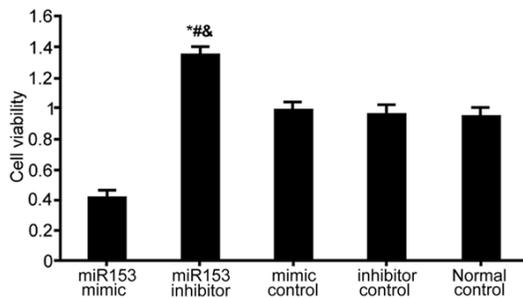


Figure 1. T24 cell viability after transfection. *P < 0.05, compared with the mimic control. #P < 0.05, compared with the inhibitor control. &P < 0.05, compared with the normal control.

Reagents and instruments

MiR-153 mimics and the miR-153 inhibitor were bought from GenePharma (Shanghai, China). DMEM medium, penicillin/streptomycin, and FBS were obtained from Gibco (USA). Lipofectamine 2000 was derived from Invitrogen (USA). MiR-153, β -actin, and a RT-PCR detection kit were provided by Takara Shuzo (Japan). Trizol was obtained from Gibeo BRL (USA). Mouse anti human Bcl-2, Mcl-1, and FOXO1 primary antibodies, and rabbit anti-mouse

Methods

Routine cell culture: T24 cells were cultured in RPMI-1640 medium and maintained at 37°C and 5% CO₂.

Cell transfection

T24 cells were cultured in a DMEM medium containing 10% FBS, 100 U/ml penicillin, and 100 U/ml streptomycin at 37°C and 5% CO₂. The cells were seeded in the dish and transfected by miR-153 mimics, an miR-153 inhibitor, and a mimic control or inhibitor control using lipofectamine 2000. After 4-6 h, the medium was changed and the cells were further cultured for 48 h for the subsequent experiments.

Real-time PCR

Total RNA was extracted from the T24 cells after transfection and quantified by calculating D260 nm/D280 nm. A total of 200 ng RNA was reverse transcribed to cDNA by synthesizing the poly A tail of miRNA. The primers used are listed in **Table 1**. The PCR reaction was performed at 95°C for 5 min, followed by 95°C for 30 s, 58°C for 30 s, and 72°C for 30 s. U6 was selected as internal reference.

MTT assay

After transfection, the cells in the logarithmic phase were seeded in the dish at 8×10^4 /well. Cell viability was evaluated at 12, 24, and 48 h. A total of 20 μ l MTT (5 mg/ml) were added to the well for 4 h. Then, 150 μ l DMSO was added to the plate for 10 min and tested at 570 nm to obtain the absorbance value. A cell viability curve was drawn.

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Table 3. Cell cycle comparison after transfection

Group	G ₀ -G ₁ (%)	S (%)	G ₂ -M (%)
miRNA-153 mimic	77.0 ± 1.5	6.5 ± 3.2 ^{*,#,&}	16.6 ± 4.0
miRNA-153 inhibitor	94.0 ± 1.5	2.5 ± 3.2 ^{*,#,&}	3.6 ± 0.2
mimic control	92.1 ± 0.5	6.5 ± 0.3	1.6 ± 0.7
Inhibitor control	92.6 ± 0.4	6.1 ± 0.4	1.8 ± 0.4
Normal control	92.4 ± 0.5	5.5 ± 0.3	2.1 ± 0.4

*P < 0.05: compared with mimic control, #P < 0.05: compared with inhibitor control, &P < 0.05: compared with normal control.

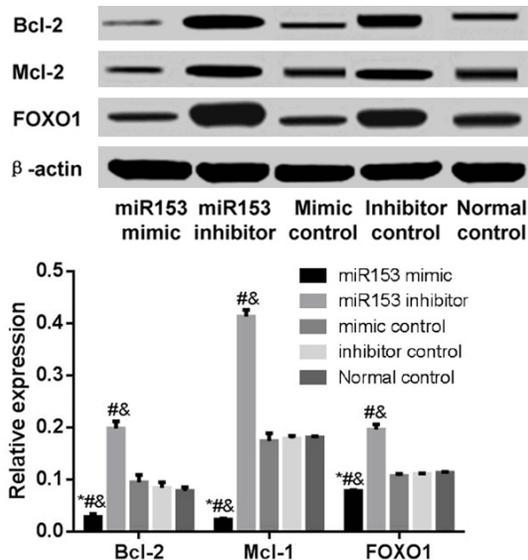


Figure 2. Bcl-2, Mcl-1, and FOXO1 protein expressions in T24 cells. *P < 0.05: compared with the mimic control, #P < 0.05: compared with the inhibitor control, &P < 0.05: compared with the normal control.

Flow cytometry

The cells were digested and centrifuged at 1000 r/min for 5 min. Then the cells were collected at 1×10^9 and fixed by 75% absolute ethanol for 12 h. Next, the cells were washed with PBS and incubated in PI at 4°C in the dark for 30 min. Finally, the cells were analyzed on a flow cytometer to evaluate the cell cycle.

Western blot

After transfection, the T24 cells in the logarithmic phase were collected to extract protein. A total of 40 µg protein was separated by 8% SDS-PAGE and blocked at room temperature for 1 h. Then the membrane was incubated in a primary antibody (1:200, β-actin 1:500) (Thermo Fisher Scientific) at 4°C overnight. Next, the membrane was incubated in a secondary anti-

body (1:2000) (Thermo Fisher Scientific) for 1 h. Finally, the membrane was treated by Western Blotting Luminol Reagent (Thermo Fisher Scientific, Waltham, MA, USA), and scanned to analyze the optical density using Quantity One software.

Statistical analysis

All data analyses were performed on SPSS 17.0 software. The measurement data were presented as the mean ± standard deviation and compared using a *t* test. The enumeration data were analyzed by ANOVA. P < 0.05 was depicted as statistical significance.

Results

Increased miR-153 expression in T24 cells after the transfection of a miR-153 mimic

To test the miR-153 expression in T24 cells after transfection, we applied RT-PCR and found that miR-153 expression increased in the miR-153 mimic group, while the miR-153 was reduced in miR-153 inhibitor group compared with the mimic control, inhibitor control, and normal control groups (P < 0.05) (Table 2).

Decreased cell viability after transfection

To assess T24 cell viability after transfection, we adopted an MTT assay and discovered that cell viability was alleviated in the miRNA-153 mimic group, whereas cell viability was enhanced in the miRNA-153 inhibitor group compared with the control following the time extension (P < 0.05) (Figure 1).

Increased cell cycle after transfection

Cell proportion in the S phase increased in the miRNA-153 mimic group, whereas it was attenuated in the miRNA-153 inhibitor group compared with the control (P < 0.05) (Table 3).

Reduced Bcl-2, Mcl-1, and FOXO1 protein expressions in T24 cells after transfection

The Bcl-2, Mcl-1, and FOXO1 proteins were downregulated in the miRNA-153 mimic group, while they were elevated in the miRNA-153 inhibitor group compared with the control (P < 0.05) (Figure 2, Table 4).

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Table 4. Bcl-2, Mcl-1, and FOXO1 protein expressions in T24 cells

Group	Bcl-2	Mcl-1	FOXO1
miR153 mimic	0.028 ± 0.006 ^{*,#,&}	0.023 ± 0.002 ^{*,#,&}	0.078 ± 0.002 ^{*,#,&}
miR153 inhibitor	0.198 ± 0.009 ^{#,&}	0.413 ± 0.013 ^{#,&}	0.196 ± 0.01 ^{#,&}
mimic control	0.095 ± 0.014	0.174 ± 0.015	0.107 ± 0.004
Inhibitor control	0.084 ± 0.011	0.179 ± 0.005	0.111 ± 0.001
Normal control	0.078 ± 0.007	0.181 ± 0.002	0.113 ± 0.002

*P < 0.05: compared with the mimic control, #P < 0.05: compared with the inhibitor control, &P < 0.05: compared with the normal control.

Discussion

Bladder cancer is one of the most common malignant tumors in our country, accounting for 3.3% [8]. The application of the theory of innovative life science and technology research on the occurrence and development mechanisms of bladder cancer and further exploring new and effective diagnosis and treatment methods are the leading edge of urology. MiRNAs are a type of endogenous small molecule single-strand RNAs with a length of about 22 nt. MiRNAs are highly conserved in evolution with a posttranscriptional regulatory function. They are coded by the intron or other noncoding region of DNA and are transcribed under the effects of RNA polymerase II. These small molecules target mRNA via the RNA induced silencing complex to block translation or guide digestion. MiRNAs have multiple biological functions, such as regulating cell development, differentiation, viability, and apoptosis [9-12]. This study selected bladder cancer cell line T24 to analyze the impact of miR-153 on cell viability and cycle.

MiR-153 expression increased in the miR-153 mimic group, while miR-153 was reduced in the miR-153 inhibitor group compared with the mimic control, inhibitor control, and normal control groups, suggesting miR-153 is positively expressed in T24 cells and suggesting a successful transfection. A previous study found that miR-153 is a tumor suppressor in ovary cancer. Furthermore, miR-153 exhibited different levels in ovary cancer tissues at different stages or subtypes, so it can be used as a biomarker for ovary cancer diagnosis and prognosis [13]. Wu reported that miR-153 was significantly increased in prostate cancer cells 22RV1 and DU145, a finding which is similar to our results [14].

This study further explored T24 cell viability after transfection. Cell viability was alleviated

in the miRNA-153 mimic group, but cell viability was enhanced in the miRNA-153 inhibitor group, revealing that miR-153 suppressed bladder cancer cell T24 viability. Cell cycle analysis demonstrated that cell proportion in the S phase increased in the miRNA-153 mimic group,

indicating that miR-153 may inhibit T24 cell viability by suppressing protein synthesis, restraining cell division, and arresting the cell cycle to facilitate cell apoptosis. It showed that miR-153 mimics transfection promoted glioma cell U251 apoptosis [15]. Meanwhile, siRNA transfection downregulated Nrf1 expression to enhance glioma U251 cell apoptosis [16].

To investigate the mechanisms of miR-153 on T24 cell viability and cycle, we detected Bcl-2, Mcl-1, and FOXO1 protein expressions in T24 cells after transfection. Bcl-2, Mcl-1, and FOXO1 proteins were downregulated in the miRNA-153 mimic group, while they were elevated in the miRNA-153 inhibitor group, indicating that miR-153 plays its role by suppressing Bcl-2, Mcl-1, and FOXO1. It was pointed out that Bcl-2 and Mcl-1 may be the target genes of miR-153. MiR-153 promoted cell apoptosis and inhibited cell viability by directly binding with the 3'-UTR of Bcl-2 and Mcl-1 mRNA to restrain expressions [17, 18]. Another study revealed that FOXO1 expression is downregulated in miR-153 highly expressed cancer cells, but miR-153 inhibitor transfection elevates FOXO1 expression and arrests the cell cycle, which is in accordance with our results [19-21].

Conclusion

MiR-153 positively expresses in bladder cancer T24 cells and suppresses cell viability and cycle by suppressing the Bcl-2, Mcl-1, and FOXO1 protein expressions. MiR-153 participates in the occurrence and development of bladder cancer, so it can be considered a new tool to improve the treatment efficacy and prognosis of bladder cancer patients in the clinic. A variety of cytokines and signaling pathways interact with each other in the process of bladder cancer tumorigenesis. Further in depth investigation of miR-153 is needed to provide a

theoretical basis for its application in the clinic.

Disclosure of conflict of interest

None.

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