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MiRNA153 induces pituitary tumor MMQ cell line apoptosis through down-regulating Skp protein expression

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Abstract. – OBJECTIVE: Pituitary tumor seriously threatens patient's life. MicroRNAs regulate cell growth and apoptosis. This study aims to investigate the effects of miRNA153 on pituitary tumor MMQ cells proliferation and apoptosis.

MATERIALS AND METHODS: Synthetic miRNA153 and control miRNA were transfected to MMQ cell line. Cell proliferation and apoptosis were tested by MTT assay and flow cytometry, respectively. Skp protein expression was detected by Western blot assay. Skp siRNA or Skp plasmid was transfected to MMQ cells transfected by miRNA153 to evaluate the influence on MMQ cell apoptosis.

RESULTS: MMQ cell proliferation was inhibited and apoptosis was enhanced after miRNA153 transfection. Skp protein level decreased in MMQ cells transfected by miRNA153. Skp interference enhanced MMQ cell apoptosis induced by miRNA153. Skp overexpression restrained MMQ cell apoptosis triggered by miRNA153.

CONCLUSIONS: MiRNA153 transfection suppressed MMQ cell growth and induced apoptosis. MiRNA153 regulated MMQ cell apoptosis through down-regulating Skp protein.

Key Words:
miRNA153, Skp protein, Pituitary tumor MMQ cell line, Apoptosis.

Introduction

Pituitary tumor is an important type of neurological tumor[1]. Though the pathological basis is still unclear, hormones abnormal secretion, aging, and genetic factors are considered to be the main decision factors[2]. As a synergy factor of pituitary tumor infection, smoking may increase the risk of pituitary tumor. Also, mental pressure and poor immune condition can affect the occurrence of the disease. Pituitary tumor brings heavy physical and mental burden to patients[3,4]. Surgery, radiotherapy, and chemotherapy are commonly used for the treatment of pituitary tumor. However, they also have many kinds of shortcomings and deficiencies, such as bleeding and other side effects[5-7]. It is an important and difficult topic to improve the accuracy and success rate of pituitary tumor treatment. Molecular targeted therapy is a new way for tumor treatment[8-10]. Its difficulty and key point are the choices of molecular targets. Moreover, it showed poor efficacy on pituitary tumor[11]. Therefore, more effective molecular targets for pituitary tumor are seriously needed in clinical application[11,12]. More importantly, it is still a lack of miRNA as targets for the treatment of pituitary tumor[12]. MiRNAs regulate multiple biological processes including cell cycle and survival. For instance, miRNA218 can suppress pituitary tumor growth, while miRNA34a is related to tumor metastasis[13,14], suggesting that miRNAs may also be involved in the occurrence and development of pituitary tumor[13-15]. Previous studies suggested that miRNA153 was significantly increased in pituitary tumor tissue compared with paracarcinoma tissue, revealing that miRNA153 may participate in the development of pituitary tumor[16,17]. This study intended to explore the potential regulatory effect of miRNA153 on pituitary tumor MMQ cells. The strategy of anti-tumor is to kill tumor cells without affecting normal cells. Apoptosis is regulated by anti-apoptotic proteins and pro-apoptotic proteins[18,19]. Ideal anti-cancer drug can reduce anti-apoptotic protein levels and up-regulate pro-apoptotic protein expressions. Skp protein is a widely investigated antiapoptotic molecule[20,21]. At present, although many drugs targeted Skp protein, their effect in reducing Skp level is still unsatisfactory[22]. This study also tried to study the potential molecules that can target Skp protein. Therefore, it took pituitary tumor cell line MMQ to discuss the role and possible mechanism of miRNA153 on pituitary tumor.

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Materials and Methods

Materials and Cell Model

MMQ cell line was purchased from ATCC cell bank (Manassas, VA, USA). Fetal bovine serum (FBS) and Dulbecco's Modified Eagle's medium (DMEM) were bought from Gibco (Rockville, MD, USA). MiRNA153 (5'-TCACTATCGGAGGTCATCCAAT-3' and 5'- AGGTGTAAAAGAAAGAACGCA-3'), scramble miRNA (5'-CCCATGGTTACCTATGAGGC-3' and 5'-TTTCGCACAATGTCAGATT-3'), Skp siRNA (5'-CTATCCATCGAGGCTAGTGC-3' and 5'-TCGATTATTCACTAAGCGT-3'), and Skp plasmid were got form GenePharma (Shanghai, China). Lipofectamine cell transfection kit was from Invitrogen (Carlsbad, CA, USA). MTT was bought form Beijing Dingguo Changsheng Biotechnology Co. Ltd. (Beijing, China). FITC-annexin, Caspase-3 detection kit, and Western blot related reagents were form Beyotime Biotechnology. Co. Ltd. (Shanghai, China). IgG mouse anti-human Skp and actin monoclonal antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Cell Culture

MMQ cells were resuscitated and cultured in high glucose Dulbecco's Modified Eagle's medium (DMEM); Gibco (Rockville, MD, USA) medium[9].

Transfection

MiRNA153 and scramble miRNA were transfected into MMQ cells using lipofectamine method. MMQ cells were seeded in 6-well plate at the density of 80%. A total of 1 μl miRNA154 or scramble miRNA at 1 μg/μl was suspended in lipo2000 and then transfected to MMQ cells[9].

MTT Assay

MMQ cell viability was tested by MTT according to the conventional method[10]. MMQ cells were seeded in 24-well plate for 12 h. After transfected by miRNA153 or scramble miRNA, the cells were added with MTT at 2 mg/ml for 4 h. Next, the reaction was stopped by DMSO for 5 min. At last, the plate was read on microplate reader at 560 nm to obtain the absorbance value[11].

Flow Cytometry

MMQ cell apoptosis was tested by flow cytometry upon Annexin-V-FITC double staining method. MMQ cells were transfected with miRNA153 or scramble miRNA and collected after 48 h. The cells in 500 μl were mixed with 100 μl buffer and 2 μl Annexin-V-FITC at room temperature avoids of light for 25 min. At last, cells were tested on flow cytometry to evaluate cell apoptosis[14].

Western Blot Assay

Total protein was extracted according to the kit instruction and quantified by BCA kit Gibco (Rockville, MD, USA). A total of 20 μg protein was boiled for 6 min and separated by electrophoresis. After transferring and blocking, the membrane was incubated in antibody. At last, the membrane was washed with tris buffered saline-tween (TBST) and developed using enhanced chemiluminescence (ECL) reagent Amersham Biosciences (Piscataway, NJ, USA). The image was obtained by gel imaging system to analyze protein expression[15].

Caspase-3 Activity Detection

Caspase-3 activity in MMQ cells was tested to evaluate cell apoptosis. After transfected by miRNA153 or scramble miRNA, MMQ cells were resuspended in Dulbecco's Modified Eagle Medium (DMEM) and added with chromophoric substrate at room temperature. Then the cells were put into 24-well plate and detected on microplate reader[17]. Caspase-3 relative activity was calculated as different value between absorbance in miRNA153 group and scramble miRNA group.

Skp Protein Interference and Overexpression

To test the impact of Skp protein interference or over-expression on MMQ cells after miRNA153 transfection, Skp siRNA or plasmid was transfected to MMQ cells upon lipo2000 according to the manual. Then miRNA153 or scramble miRNA was further transfected to MMQ cells. MMQ cells were seeded 24-well plate at the density of 70%. A total of 2 μl Skp siRNA or plasmid at 0.5 μg/μl was suspended in lipo2000 and transfected to MMQ cells. Next, a total of 4 μl miRNA154 or scramble miRNA at 1 μg/μl was suspended in lipo2000 and then transfected into MMQ cells transfected by Skp siRNA or plasmid.

Statistical Analysis

All data analysis was performed on SPSS 11.0 software (SPSS Inc. Chicago, IL, USA). The data was presented as mean ± standard deviation and compared by *t*-test. $p<0.05$ was depicted as statistical significance.

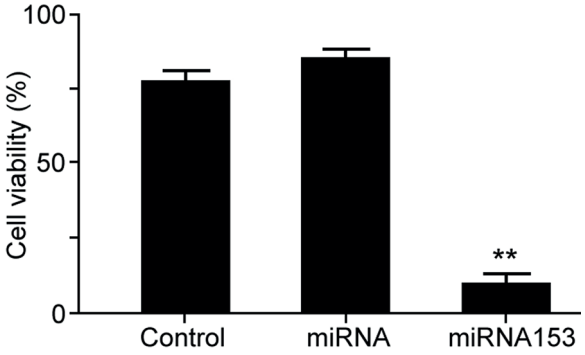

Figure 1. MiRNA153 suppressed MMQ cell viability. $^{**}p<0.01$ compared with miRNA group.

Results

MiRNA153 Suppressed MMQ Cell Viability

MTT assay results showed that compared with MMQ cells transfected by 0.5 μg scramble miRNA, MMQ cell viability significantly decreased after 0.5 μg miRNA153 transfection (p=0.0047) (Figure 1). Since no statistical difference was observed in cell viability between scramble miRNA transfection and normal control (p>0.05), cells transfected with scramble miRNA were treated as control in the following experiments.

MiRNA153 Induced MMQ cell Apoptosis

Annexin-V-FITC staining method was applied to test MMQ cell apoptosis. It was revealed that phosphatidylserine eversion in MMQ cells transfected by 1 μg miRNA153 was enhanced compared to MMQ cells transfected by 1 μg scramble miRNA (p=0.0082) (Figure 2).

MiRNA153 Activated Caspase-3 in MMQ Cells

As shown in Figure 3, caspase-3 activity in MMQ cells transfected by 0.5 μg miRNA153 was markedly higher than that in MMQ cells transfected by scramble miRNA (p=0.029).

MiRNA153 Reduced Skp Protein Expression in MMQ cells

Western blot was adopted to determine Skp protein expression in MMQ cells. As shown in Figure 4, Skp protein level in MMQ cells transfected by 0.5 μg miRNA153 was significantly lower than that in MMQ cells transfected by scramble miRNA (p=0.056).

Skp Knockdown Enhanced MMQ Cell Apoptosis Induced by miRNA153

To evaluate the influence of Skp protein on miRNA153 induced MMQ cell apoptosis, Skp siRNA was transfected to MMQ cells to observe the phenomenon. Western blot results demonstrated that Skp level declined after Skp siRNA transfection. Skp protein expression in Skp siRNA + miRNA153 group was lower than that in miRNA153 group (Figure 5A). Caspase-3 detection exhibited that caspase-3 activity apparently enhanced in Skp siRNA + miRNA153 group compared with miRNA153 group (p=0.026) (Figure 5B).

Skp Protein Overexpression Suppressed MMQ Cell Apoptosis Induced by miRNA153

To determine the impact of Skp protein on miRNA153 induced MMQ cell apoptosis, Skp plasmid was transfected to MMQ cells to overexpress Skp. Western blot revealed that Skp level up-regulated after Skp plasmid transfection. Skp protein expression in Skp + miRNA153 group was significantly higher than that in miRNA153 group (Figure 6A). Caspase-3 detection suggested

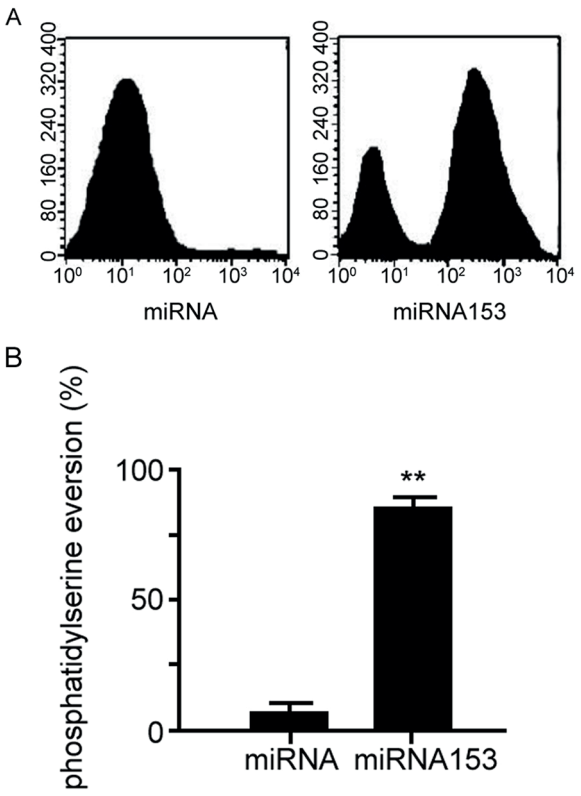

Figure 2. MiRNA153 induced MMQ cell apoptosis. $^{**}p<0.01$ compared with miRNA group.

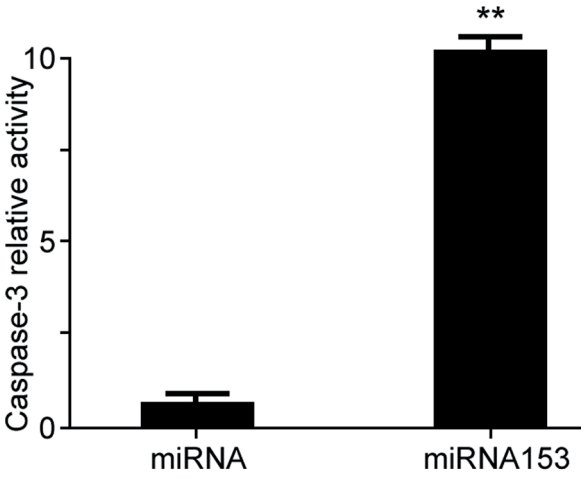

Figure 3. MiRNA153 activated Caspase-3 in MMQ cells. $^{*}p<0.05$ compared with miRNA group.

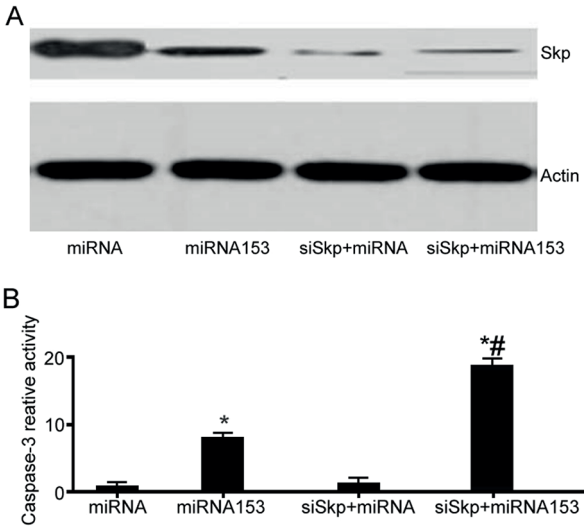

Figure 5. Skp knockdown enhanced MMQ cell apoptosis induced by miRNA153. $^{*}p<0.05$ compared with miRNA group. $^{\#}p<0.05$ compared with miRNA153 group.

that caspase-3 activity apparently declined in Skp + miRNA153 group compared with miRNA153 group (p=0.022) (Figure 6B).

Discussion

This study investigated the regulatory role and the possible mechanism of miRNA153 on pituitary tumor MMQ cells from molecule and protein levels. It was showed that miRNA153 transfection reduced MMQ cell viability, suppressed cell growth, and induced cell apoptosis. It was consistent with previous report, as that miRNA participated in cell growth and survival[3]. There is still a lack of study to investigate the impact of miRNA to the pituitary tumor[3]. Therefore, it has both the theoretical significance and practical value to explore miRNA regulation of tumor cell growth and survival. Skp protein is an anti-apoptotic protein[23]. It is still controversy whether Skp protein is regulated by miRNA153 to mediate MMQ cell growth[24,25]. Our results demonstrated that the miRNA153 transfection reduced Skp protein level in MMQ cells. MMQ cell apoptosis increased after miRNA153 transfection and Skp protein knockdown, while it reduced after Skp plasmid transfection. In this study, three different results proved the role of Skp protein in pituitary tumor MMQ cell apoptosis induced by miRNA153. Skp protein declined in MMQ cells after miRNA153 transfection. Skp interference enhanced MMQ cell apoptosis induced by miRNA153. Skp over-expression restrained MMQ cell apoptosis triggered by miRNA153. It suggested the role of Skp protein in MMQ apoptosis induced by miRNA153. Moreover, targeting Skp might be a new strategy for pituitary tumor treatment[26]. At present, Skp protein also plays an anti-apoptotic role in other types of cancer cells[22,25,27,28]. However, there is still no report about the regulation between miRNA153 and Skp protein in pituitary tumor. This study also had the following deficiencies: firstly, we did not collect the clinical specimen of pituitary tumor, thus can't discuss the rela-

Figure 4. MiRNA153 reduced Skp protein expression in MMQ cells.

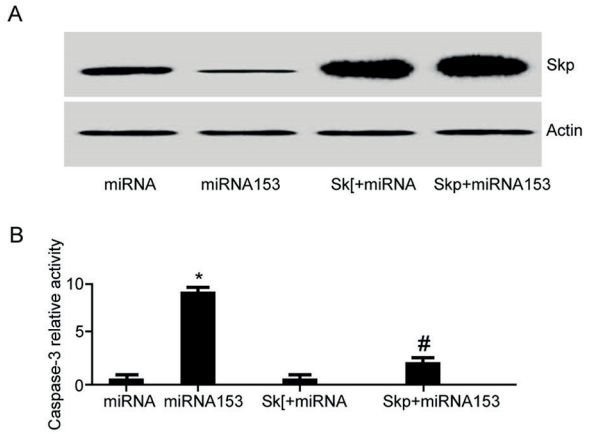

Figure 6. Skp over-expression reduced MMQ cell apoptosis induced by miRNA153. ***(A)*** Western blot detection of Skp protein expression. ***(B)*** Caspase-3 relative activity. $^{*}p<0.05$ compared with miRNA group. $^{\#}p<0.05$ compared with miRNA153 group.

tionship between Skp protein and pituitary tumor in clinic; secondly, we did not obtain the pituitary tumor tissue from patients received therapy, thus we can't observe Skp protein level changes after treatment to confirm the role of Skp in pituitary tumor; lastly, we did not establish the pituitary tumor animal model to observe the curative efficacy of targeting miRNA153 on pituitary tumor *in vivo*.

Conclusions

MiRNA153 transfection suppressed pituitary tumor MMQ cell viability. MiRNA153 induced MMQ cell apoptosis through down-regulating Skp protein level. It suggested that Skp might be a potential treatment target for pituitary tumor, which may provide theoretical basis for its application.

Conflict of interest

The authors declare no conflicts of interest.

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