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MiR-34a affects G2 arrest in prostate cancer PC3 cells *via* Wnt pathway and inhibits cell growth and migration

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Abstract. – OBJECTIVE: To investigate the effect and mechanism of miRNA-34a overexpression on proliferation and migration of PC3 prostate cancer cells.

PATIENTS AND METHODS: Benign prostatic hyperplasia tissue (30 cases), prostate cancer tissue (30 cases), and prostate paracancerous tissue (30 cases) were collected. Levels of miRNA-34a in these fresh tissues were measured by fluorescence quantitative PCR. PC3 cells were divided into non-loaded group and overexpression group. Cells in the non-loaded group were transfected with non-loaded plasmid. Cells in the overexpression group were transfected with miRNA-34a plasmid, and the miRNA-34a level was determined by fluorescence quantitative PCR to confirm the overexpression. Cell proliferation was analyzed by CCK-8 assay. Cell migration rate was measured by cell scratch assay. Flow cytometry was used to detect apoptosis and analyze cell cycle. Western blot was used to measure the expression levels of β-catenin, E-cadherin and Vimentin.

RESULTS: The expression level of miRNA-34a in prostate cancer tissue was significantly lower than that in prostate paracancerous tissue. Dual-Luciferase reporter gene assay was used to analyze the transcriptional activity of Wnt1 gene. The proliferation and migration of PC3 cells were significantly decreased after overexpression of miRNA-34a, and the differences were statistically significant compared with those in the non-loaded group ($p<0.05$). Flow cytometry analysis showed that in the overexpression group, the apoptotic rate, as well as the proportion of cells in the G2 phase, was significantly higher than that in the non-loaded group ($p<0.05$). The β-catenin level in the nucleus of PC3 cells was significantly reduced after overexpression of miRNA-34a. The total protein levels of β-catenin and Vimentin were significantly decreased, whereas the level of E-cadherin in the overexpression group was apparently increased, compared with that in the non-loaded group. The Dual-Luciferase reporter gene showed a decrease in the relative fluorescence intensity of Wnt1 after overexpression of miR-34a ($p<0.05$).

CONCLUSIONS: Overexpression of miRNA-34a inhibits Wnt/β-catenin pathway by regulating the transcriptional activity of Wnt1, thereby regulating the proliferation and migration of PC3 cells and promoting apoptosis.

Key Words:
Prostate cancer, MiRNA-34a, β-catenin, Epithelial-mesenchymal transition, Proliferation.

Introduction

Prostate cancer is the most common primary tumor in the male urinary system, accounting for 18% of all urological tumor types[1]. Current treatments for prostate cancer are mainly surgery and postoperative chemotherapy. For some patients who received surgical treatment, their postoperative survival time is relatively short, and the recurrence rate is high[2]. The pathogenesis of prostate cancer has not yet been fully elucidated. It was reported that ionizing radiation, lifestyle and genetic factors are the main causes and risk factors of prostate cancer[3]. With the advances in science and technology and further understanding of the differential genes and molecular mechanisms of prostate cancer, the search for molecules that regulate prostate cancer has become a new direction for cancer treatment.

A miRNA is a small non-coding RNA molecule. Its translation and degradation can be controlled through binding to related mRNA downstream proteins, so as to regulate biolog-

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ical processes such as proliferation, differentiation and migration of tumor cells[4,5]. Many studies[6-8] showed that miRNAs played important roles in modulating proliferation, migration and invasion of prostate cancer cells. It was also found that miRNAs were associated with tumor sensitivity to chemotherapy. Recently, miR-34a was reported to have a potential effect as a tumor suppressor in prostate cancer[9]. However, the underlying mechanism was not fully understood. The protein β-catenin not only plays an important role in cell adhesion, but also acts as a key molecule in the Wnt signaling pathway[10,11]. The Wnt signaling pathway is involved in virtually every aspect of embryonic development. Therefore, its abnormal activation may result in onset of malignant tumors. It was confirmed in multiple studies that the Wnt/β-catenin signaling pathway played an important role in prostate cancer[12]. The Wnt/β-catenin signaling pathway is regulated by a variety of miRNAs[13]. So far, the role of miRNA-34a in regulating the Wnt/β-catenin signaling pathway in prostate cancer was not reported. In this study, the expression of miRNA-34a in prostate cancer was investigated. Meanwhile, PC3 cells were transfected with miRNA-34a overexpression plasmid to detect the effects of miRNA-34a on PC3 cell proliferation and migration by regulating the Wnt/β-catenin signaling pathway.

Patients and Methods

Materials and Reagents

The following materials and reagents were purchased from commercial sources: PC3 cells from the Shanghai Cell Bank Affiliated with Chinese Academy of Sciences; the TRIzol reagent and Lipofectamine 2000 transfection reagent from Invitrogen (Carlsbad, CA, USA); the dyes for fluorescence quantitative PCR from Bio-Rad (Hercules, CA, USA); the reverse transcription kits from Toyobo (Chiyoda-ku, Tokyo, Japan); the Dulbecco's Modified Eagle's Medium (DMEM) high glucose medium and fetal bovine serum from Gibco (Carlsbad, CA, USA); the CCK-8 kit (product number: C0037) and bicinchoninic acid (BCA) kit (product number: P0009) from Beyotime Biotechnology (Shanghai, China); the cell cycle assay kit from MultiSciences (Hangzhou, Zhejiang, China); the apoptosis detection kit from Sungene (Tianjin, China); the anti-Vimentin antibody (product number: ab92547), anti-E-cadherin antibody (product number: ab40772), and anti- β-catenin antibody (product number: ab32572) from Abcam (Cambridge, MA, USA); the internal reference β-actin antibody (product number: 20536-1-AP), horseradish peroxidase (HRP)-labeled secondary antibody (product number: SA00001-2) and FITC-labeled secondary antibody (product number: SA00003-2) from Proteintech (Wuhan, Hubei, China); miR-34a overexpression, empty plasmid and firefly fluorescent vector pGL3 promoter gene and sea kidney fluorescent vector pRL-SV40 were synthesized by RiboBio (Guangzhou, Guangdong, China), and Dual-Luciferase assay kit was provided by Progema (Romanore, Mantova, Italy). Flow cytometry was performed on a Beckman CytoFLEX flow cytometer (Beckman Coulter, Boulevard Brea, CA, USA).

Patients

Tissues were collected from 30 patients who underwent radical prostatectomy in The Second Affiliated Hospital of Qiqihar Medical University. Inclusion criteria: (1) patients with confirmed prostate cancer by postoperative pathological test; (2) patients with confirmed benign prostatic hyperplasia by pathological test regardless of the stage. Exclusion criteria: (1) patients whose prostate cancer metastasized to neighboring organs, lymph nodes or distant organs; (2) patients who had big cancer lesions; (3) patients whose paracancerous tissue was unable to collect. The standards refer to the 2016 edition clinical guidance for screening, diagnosis, and local treatment of prostate cancer (PCa) from the European Association of Urology (EAU) – European Society for Radiotherapy & Oncology (ESTRO) - International Society of Geriatric Oncology (SIOG)[14,15]. The selected patients aged from 53 to 64 years with an average age of 58 ± 8.9 years. All patients were aware of the use of the specimen before surgery and signed an informed consent form. This investigation\ was approved by the Medical Ethics Committee of The Second Affiliated Hospital of Qiqihar Medical University. The cancer tissue and the paracancerous tissue 1 cm away from the tumor edge were collected under observation of naked eyes. One portion of the tissue was stored in liquid nitrogen and the other portion was sent for pathological test. After pathological test, 30 samples were confirmed to be cancer tissues, and 30 samples were confirmed to be paracancerous tissues.

RNA Extraction and Fluorescence Quantitative PCR

RNA was extracted from homogenized prostate cancer tissue and paracancerous tissue using TRIzol reagent. Total RNA in PC3 cells was extracted using total RNA extraction kit manufactured by Magen Tech (product number: R4111-01). The RNA concentration was measured by spectrophotometer. Complementary DNA (cDNA) was synthesized from 1 μg of RNA via reverse transcription in accordance with the manual coming with the reverse transcription kit. The PCR amplification reaction was performed as follows: pre-denaturation at 95°C for 5 min, 40 cycles of 95°C for 5 s (denaturation), 60°C for 30 s (anneal), and 72°C for 30 s (extension), followed by extension at 72°C for 5 min at the end of the cycles. U6 was used as the internal reference gene. The primer sequences were as follows: for U6 the forward sequence was GGA ACG ATA CAG AGA AGA TTA GC, and the reverse sequence was TGG AAC GCT TCA CGA ATT TGC G; for miR-34a the forward sequence was ACA CTC CAG CTG GGT GGC AGT GTC TTA G, and the reverse sequence was CTC AAC TGG TGT CGT GGA GTC G. All samples were run in triplicate. The relative expression level of the target gene was calculated using $2^{-\Delta\Delta Ct}$ method, where $\Delta Ct=Ct_{target\ gene}-Ct_{internal\ reference}$ and $\Delta\Delta Ct=\Delta Ct_{experimental\ group}-\Delta Ct_{control\ group}$.

Cell Grouping and Transfection

PC3 cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS) in a humidified incubator supplied with 5% CO_2 and maintained at 37°C. The medium was changed every other day, and the cells were passaged at about 80% confluency. The cells were seeded in a 6-well plate and divided into non-loaded group and overexpression group. Cells in the non-loaded group were transfected with non-loaded plasmid, and cells in the overexpression group were transfected with miRNA-34a plasmid. Transfection was performed when the cells were about 30-50% confluent. The transfection protocol was briefly described below. 250 μL of opti-MEM medium was added to 4 μg of plasmid. Another 250 μL of opti-MEM medium was added to 10 μL of Lipofectamine 2000. After incubation at room temperature for 5 min, the two solutions were combined, mixed and incubated for another 5 min. This transfection mixture was added to cells in the 6-well plates. After 4-6 h of incubation, the medium was replaced with complete medium.

Cell Proliferation Assay

The transfected PC3 cells were harvested during the logarithmic phase of growth and digested with 0.25% trypsin. The cells were re-suspended in medium and seeded into a 96-well plate at 3×10^3 cells/well in 200 μL. The assay was run in sextuplicate and a blank control in each group. The cells were cultured for 5 days, during which cell proliferation was measured every 24 h. Specifically, 20 μL of the CCK-8 reagent was added to each well, followed by measurement of the absorbance at a wavelength of 450 nm using a microplate reader after incubation at 37°C for 2 h.

Cell Scratch Assay

The cells were seeded into a 6-well plate at $3x10^5$ cells/well. When cells were 100% confluent, the medium was removed and replaced with serum-free medium. After starvation for 24 h, at least three even scratches were created in the bottom of each well using a pipette tip in a perpendicular way. The plate was rinsed three times with phosphate-buffered saline (PBS) to remove detached cells, followed by serum-free medium and incubation for 24 h. Images were taken at 0 h and 24 h under a microscope to measure the widths of the scratches. Cell migration rate = (scratch width at 0 h-scratch width at 24 h)/ scratch width at 0 h.

Assessment of Cell Cycle and Apoptosis by Flow Cytometry

Cells were seeded in a 6-well plate at a density of 3×10^5 cells/well. After incubation for 24 h, the cells were harvested and centrifuged at 1200 rpm. Supernatant was discarded. After washing with PBS, the cells were subjected to flow cytometry assay in accordance with the protocol coming with apoptosis detection kit. First the cells were washed with 1 mL of 1× binding buffer. After centrifugation at 1200 rpm, supernatant was discarded, and the cell pellet was re-suspended in 1x binding buffer. The cell concentration was adjusted to 1×10^6 cells/mL. 100 μL of the cell suspension in each group was added to 5 μL of Annexin V-FITC. After mixing, the cells were incubated in the dark for 10 min at room temperature. Then, 5 μL of PI solution was added to the cells and mixed well, followed by incubation in the dark for 5 min at room temperature. The total volume was

adjusted to 500 μL by adding PBS. After mixing, the sample was analyzed on a flow cytometer. The apoptotic rate was the sum of percentages of early apoptotic cells (lower right quadrant) and late apoptotic cells (upper right quadrant). For cell cycle analysis, 1 mL of propidium iodide (PI) solution and 10 μL of permeabilization buffer were added to the cells. After incubation in the dark for 30 min at room temperature, the cells were analyzed on the flow cytometer.

Immunofluorescence Microscopy

Cells were seeded in a 24-well plate containing a glass coverslip in each well at a density of $1x10^4$ cells/well. The plate was incubated for 24 h, allowing cells to grow on the coverslips. After media removal and PBS washing, 4% paraformaldehyde was added, followed by fixation at room temperature for 10 min. After washing 3 times with phosphate-buffered saline (PBS), 0.3% Triton X-100 in PBS was added, followed by permeabilization at room temperature for 20 min. The cells were washed three times with PBS; in each wash the cells were let sit in PBS for 3 min. Then, 10% goat serum was added dropwise to the coverslips, and blocked at room temperature for 1 h. The β-catenin primary antibody was added, followed by incubation at 4°C overnight. Then, the FITC-labeled fluorescent secondary antibody was added, followed by incubation at room temperature for 1 h. After cell nuclei were stained with DAPI for 10 min, an anti-quench reagent was applied to seal the coverslips. After drying, the coverslip was mounted to a fluorescent microscope, and images were taken for analysis.

Protein Extraction and Western Blot

Treated cells were digested with 0.25% trypsin, followed by centrifugation. Appropriate amounts of RIPA lysis buffer and protease inhibitor were added to the cell pellet. For complete lysis, the cells were exposed to ultrasound on ice for 5 min. The mixture was then centrifuged at 12000 g for 15min in a low temperature centrifuge. The supernatant was collected, and 10 μL was used for concentration measurement using the bicinchoninic acid assay (BCA) method. The remaining supernatant was mixed with 5× loading buffer at a volume ratio of 4:1. The solution was boiled in a 100°C water bath for 10 minutes. Samples containing equal amount of total proteins were loaded on gels containing 5% stacking gel and 10% separation gel for electrophoresis. Electrophoresis was run at a constant voltage of 80 V until the bromophenol blue dye reached the separation gel, when the voltage was changed to 120 V until the target strips were separated. The protein strips were transferred to a polyvinylidene difluoride (PVDF) membrane by wet transfer method at a constant current of 275 mA in 80 min. After the membrane was blocked with 5% milk at room temperature for 2 h, a diluted antibody (dilution factor 1:1000 for anti-Vimentin antibody, anti-E-cadherin antibody, anti-β-catenin antibody, and anti-β-actin antibody) was added. After incubation at 4°C overnight, the membrane was washed, followed by incubation with a corresponding secondary antibody in 2% milk (dilution factor 1:10000) at room temperature for 1 h. The membrane was soaked in a developing solution to allow blots to develop. The gray value of the strips was analyzed using Image J software. B-actin was used as an internal reference. Protein expression level was the ratio of gray values of the target protein to β-actin.

Dual-Luciferase Reporter Gene Assay

The 3'UTR binding site of Wnt1 was cloned into the downstream of the pGL3 promoter vector of the Luciferase reporter vector. The reporter gene vector and miR-34a or the empty plasmid were transferred into PC3 cells according to Section 2.4 and cultured for 48 hours. The cells were lysed according to the instructions of the kit. 10 ml of the lysate and 50 ul of Luciferase assay reagent LARII were added, and the firefly fluorescence intensity RLU1 was detected after mixing. Then, 50 ul of Stop&GlO® reagent was added, the mixture was placed in a fluorescence detector to read the Renilla Luciferase intensity RLU2, and the Renilla Luciferase intensity was used as an internal reference. The transcriptional activity of Wnt1 was RLU1/RLU2.

Statistical Analysis

Data were expressed as mean ± standard deviation. Statistical analysis was performed using SPSS 20 software (IBM, Armonk, NY, USA). For comparison more than two groups, one-way analysis of variance was carried out first to find out if there were significant differences. If significant differences were found among the groups, the paired *t*-test would be used for statistical analysis. For comparison between two groups, the paired *t*-test analysis was performed. There was a statistically significant difference if $p<0.05$. Each test was repeated more than three times.

Results

Low Expression of MiR-34a in Prostate Cancer Tissue

Using fluorescence quantitative PCR, the expression of miR-34a in prostate cancer tissue was assessed and compared with that in prostate paracancerous tissue. As shown in Figure 1a, there was no significant difference in the expression of miR-34a between benign prostatic hyperplasia tissue and prostate paracancerous tissue ($p>0.05$). However, the miR-34a level in prostate cancer tissue was significantly lower than that in prostate paracancerous tissue ($p<0.05$).

Overexpression of MiR-34a Inhibits Proliferation and Migration of PC3 Cells

After transfection of PC3 cells with plasmid expressing miR-34a, the level of miR-34a mRNA in the cells was increased by 4.7-fold (Figure 1b). Overexpression of miR-34a significantly inhibited cell growth (Figure 1c), as well as cell migration rate (Figure 1d). The difference was statistically significant ($p<0.05$).

Overexpression of MiR-34a Promotes Apoptosis of PC3 Cells and Induces G2 Arrest in Cells

After transfection of PC3 cells with plasmid expressing miR-34a, the apoptotic rate (lower right quadrant + upper right quadrant) increased significantly ($p<0.05$) from 3.65±0.46 to 20.35±1.36, and the increase was mainly due to late apoptosis (Figures 2a and 2b). Compared with the non-loaded group, the percentage of cells in the S phase was significantly lower, while the percentage in the G2 phase was significantly higher in the overexpression group. The differences were statistically significant ($p<0.05$). This finding suggested a cell cycle arrest occurred at the G2 phase and was positively correlated with the apoptosis (Figures 2c and 2d).

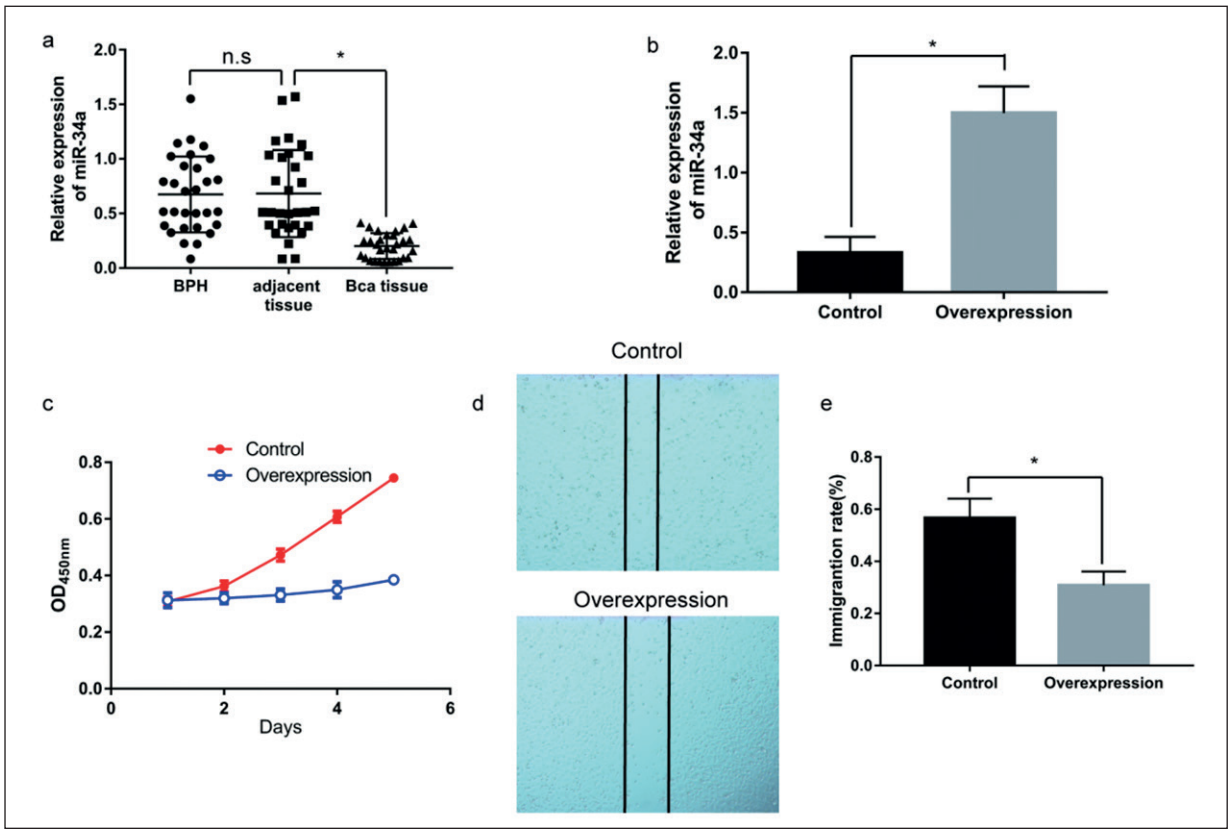

Figure 1. Expression of miR-34a in prostate cancer tissue and its effect on cell proliferation and migration. **a,** Low expression of miR-34a in prostate cancer tissue; **b**, increased expression of miR-34a in PC3 cells in the overexpression group; **c**, attenuated cell proliferation in the overexpression group; **d**, reduced cell migration in the overexpression group; e. cell mobility. Note: n.s.: no significant difference; *$p<0.05$. Control is the non-loaded group transfected with non-loaded plasmid, and Overexpression is the overexpression group transfected with plasmid expressing miRNA-34a.

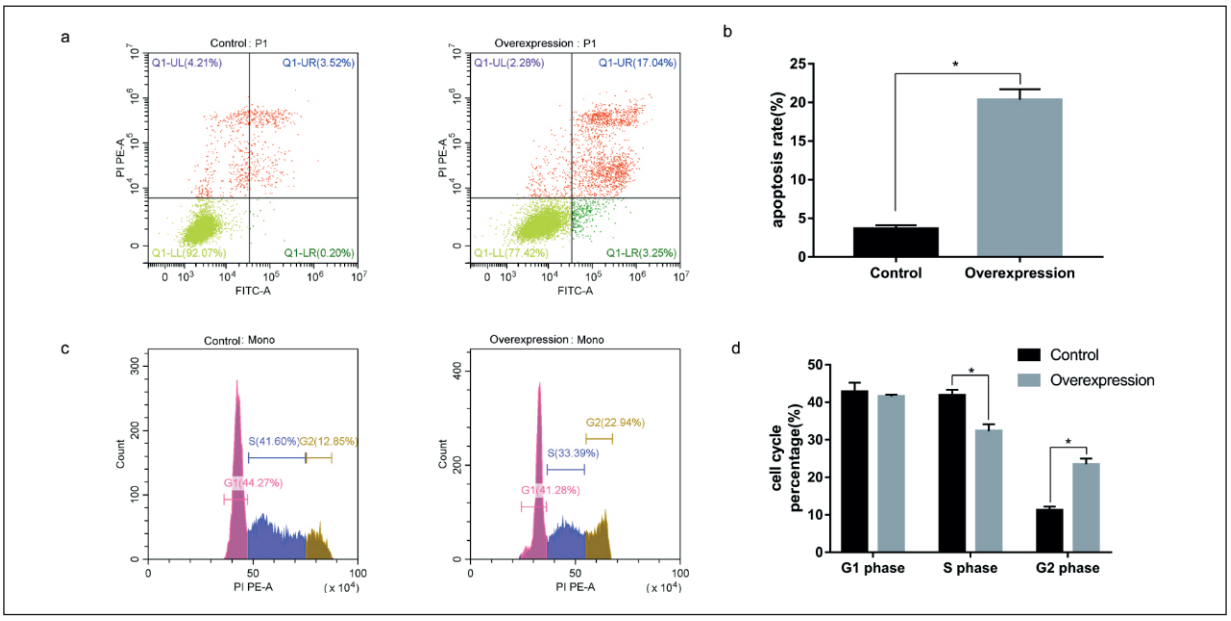

Figure 2. Effect of miR-34a overexpression on apoptosis and cell cycle. **a**/**b**, Apoptotic rate was significantly increased in the miR-34a overexpression group (Q1-UL: bare nuclei; Q1-LL: normal cells; Q1-LR: early apoptotic cells; Q1-UR: late apoptotic cells; Apoptotic rate = early apoptotic rate + late apoptotic rate); **c**, Flow cytometry analysis of cell cycle (G1, S, and G2 represent G1 phase, S phase and G2 phase, respectively); **d**, In miR-34a overexpression group, the percentage of cells in the G2 phase was increased significantly (*p<0.05). Control is the non-loaded group transfected with non-loaded plasmid, and Overexpression is the overexpression group transfected with plasmid expressing miRNA-34a.

Overexpression of MiR-34a Inhibits β-catenin Nuclear Migration and Epithelial-Mesenchymal Transition in PC3 Cells

As shown in Figure 3, in the non-loaded group, β-catenin was mainly located in PC3 cell nuclei. After PC3 cells were transfected with miR-34a, the protein was expressed mainly in the cytoplasm and very little in the nuclei. Western blot analysis showed that the level of β-catenin was significantly decreased after cells were transfected with miR-34a (Figure 4a). The difference was

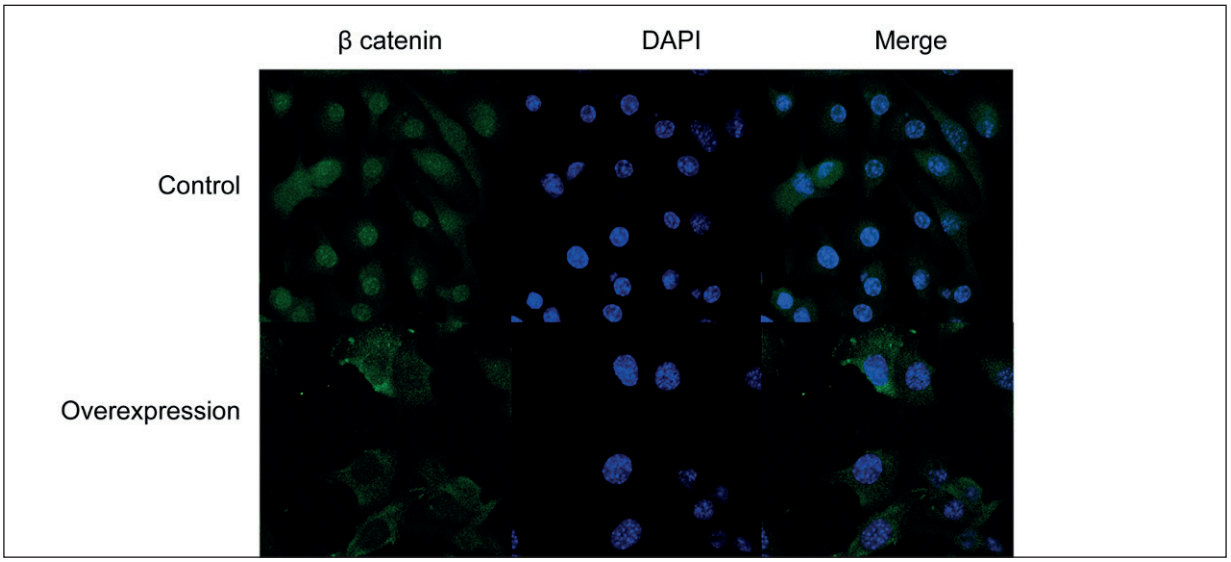

Figure 3. Immunofluorescence analysis of β-catenin localization. Control is the non-loaded group transfected with non-loaded plasmid, and Overexpression is the overexpression group transfected with plasmid expressing miRNA-34a (400 ×).

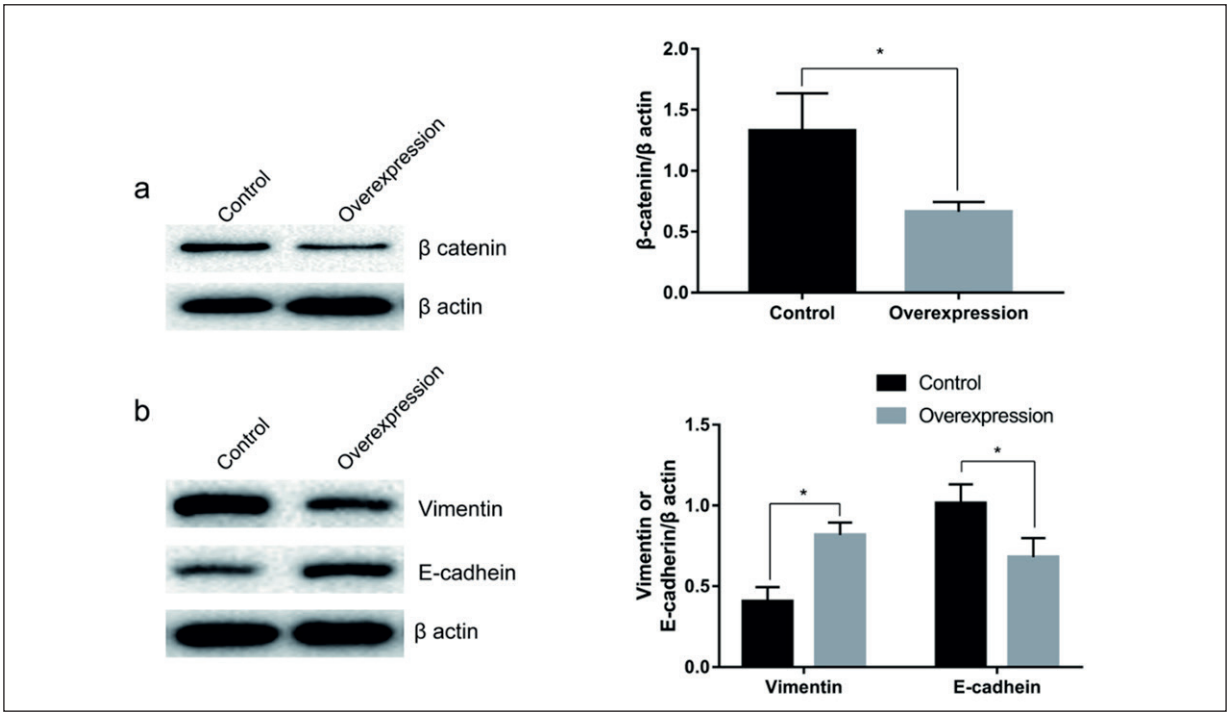

Figure 4. Western blot analysis. **a**, Effect of miR-34a overexpression on level of β-catenin; **b**, Effect of miR-34a overexpression on levels of E-cadherin and Vimentin (*p<0.05, compared with the non-loaded group). Control is the non-loaded group transfected with non-loaded plasmid, and Overexpression is the overexpression group transfected with plasmid expressing miRNA-34a.

statistically significant (p<0.05). Compared with the non-loaded group (Figure 4b), after the cells were transfected with miR-34a, the expression level of Vimentin was significantly decreased, while the level of E-cadherin was significantly increased (p<0.05).

Overexpression of MiR-34a Inhibits Wnt1 Gene Expression

As shown in Figure 5, the relative fluorescence intensity (RLU1/RLU2) of the non-loaded group was (0.84±0.13) after transfection of the Luciferase reporter gene, and the relative fluorescence intensity was (0.65±0.08) after overexpression of miR-34a. Compared with the non-load group, the relative fluorescence intensity was significantly decreased after overexpression of miR-34a, indicating that the transcriptional activity of the Wnt1 gene was significantly inhibited.

Discussion

A miRNA is a non-coding RNA molecule widely found in eukaryotic cells. Its regulation of target genes is mainly achieved by post-transcriptional degradation of mRNA or inhibition of mRNA translation[16,17]. In this study, it was found that the miR-34a level in prostate cancer tissue was significantly lower than that in prostate paracancerous tissue, suggesting that miR-34a may be associated with occurrence of

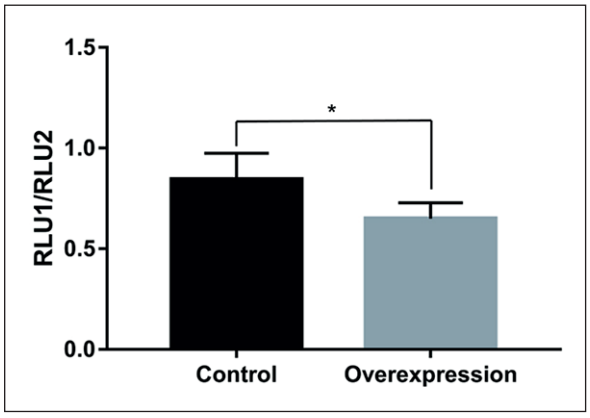

Figure 5. Dual-Luciferase reporter gene. Control is the non-loaded group transfected with non-loaded plasmid, and Overexpression is the overexpression group transfected with plasmid expressing miRNA-34a. The expression of Wnt1 gene was expressed by relative fluorescence intensity (RLU1/RLU2).

cancer. After PC3 cells were transfected with plasmid expressing miR-34a, it was found that cell proliferation was significantly attenuated. Cell scratch assay showed that cell migration rate in the overexpression group was significantly lower than that in the non-loaded group. These findings suggested that miR-34a inhibited proliferation and migration of the cancer cells through certain mechanism. Cell cycle and apoptosis were assessed as well after the cells were transfected with miR-34a. The results showed that apoptosis was significantly promoted, and the percentage of cells in the G2 phase was significantly increased, indicating a G2 arrest. Above findings suggested that miR-34a not only inhibited the proliferation and migration of PC3 cells, but also promoted apoptosis and induced G2 arrest in cell cycle.

Avtanski et al[18] reported that miR-34a was associated with the Wnt/β-catenin signaling pathway, and miR-34a played a pivotal role in cancer progression. The Wnt/β-catenin signaling pathway is a canonical Wnt signaling pathway. When it is not activated, there is no Wnt signal in cells, and β-catenin is phosphorylated by the β-catenin destruction complex assembled by APC/GSK-3β/CKIα/Axin, leading to degradation by ubiquitination. When the pathway is activated, Wnt interacts with various proteins to reduce the stability of the complex, and β-catenin accumulates in the cytoplasm. Then, excessive β-catenin translocates into the nucleus where it causes transcription of downstream target genes[19]. Therefore, activation of the Wnt/β-catenin signaling pathway plays an important role in proliferation, apoptosis, cell cycle and onset/progression of cancer cells. It was found that the level of β-catenin was significantly reduced after the cells were transfected with miR-34a. Its expression level in the nucleus was reduced as well. These findings suggested that miR-34a may inhibit the Wnt/β-catenin signaling pathway. Si et al[20] found that Wnt1 is a direct target of miR-34a and is regulated by miR-34a through the 3'UTR binding site of Wnt1. When the 3'UTR binding site is mutated, there was no change in the expression of the Wnt1 gene when the expression of miR-34a is changed. We found that Wnt1 is indeed a target of miR-34a by the Dual-Luciferase reporter gene, and the transcriptional activity of Wnt1 is inhibited after overexpression of miR-34a.

Epithelial-mesenchymal transition (EMT) plays an important role in invasion and metastasis of prostate cancer. Excessive activation of EMT in prostate cancer is manifested by increased mesenchymal cell phenotypes such as Vimentin and N-cadherin, as well as decreased epithelial phenotypes such as N-cadherin[21]. EMT in cancer is modulated by the Wnt/β-catenin signaling pathway. Activation of the Wnt/β-catenin signaling pathway modulates the expression of snail and ZEB1, thereby promoting EMT[22,23]. In this study, PC3 cell migration rate was found to be significantly reduced after the cells were transfected with miR-34a. It was also found that the level of mesenchymal cell phenotype Vimentin was significantly reduced, while the level of E-cadherin was significantly increased. Alterations of Vimentin and E-cadherin levels may result from attenuated EMT caused by miR-34a inhibition of the Wnt/β-catenin signaling pathway.

Conclusions

In summary, we found that overexpression of miR-34a inhibits the wnt/βcatenin signaling pathway by regulating the transcriptional activity of Wnt1, then prostate cancer cell proliferation was attenuated, and apoptosis was promoted. In addition, inhibition of the Wnt/β-catenin signaling pathway could reduce EMT by modulating expression of EMT-related genes and proteins, thereby reducing the migration rate of cancer cells. In conclusion, the regulatory role of miR-34a in the Wnt/β-catenin signaling pathway was confirmed in prostate cancer. However, the mechanism of miR-34a's regulation of the Wnt/β-catenin signaling pathway needs further study. Some of the results of this study disagree with other reports. In cancer cells the Wnt/β-catenin signaling pathway was over-activated, resulting in translocation of a large amount of β-catenin into the nucleus. According to literature, excessive β-catenin in the nucleus promoted expression of the target gene cyclinD1, thereby inducing G1 arrest in cell cycle[24]. However, in this study, G2 arrest was found after the cells were transfected with overexpression of miR-34a, which was not in line with the above literature report. It was speculated that the cell cycle arrest found in this study was not directly regulated by β-catenin. Instead, miR-34a may regulate the expression of other genes, leading to G2 arrest in cell cycle[25,26]. The underlying mechanism needs further study.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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