Abstract. – OBJECTIVE: To explore the role of microRNA-19a-3p in regulating invasion, metastasis and EMT (epithelial mesenchymal transition) of prostate cancer (PCa) cells, as well as its underlying mechanism.

MATERIALS AND METHODS: MicroRNA-19a-3p mimic and negative control plasmid were first constructed. After transfection of microRNA-19a-3p mimic or negative control in DU145 cells, expression levels of microRNA-19a-3p and SOX4 were detected by quantitative Real-time-polymerase chain reaction (qRT-PCR) and Western blot. The regulatory effects of microRNA-19a-3p on migration and invasion of DU145 cells were detected by wound healing assay and transwell assay, respectively. Protein levels of matrix metalloproteinase-2 (MMP2), matrix metalloproteinase-9 (MMP9), N-cadherin, Vimentin, alpha-smooth muscle actin (α-SMA) and E-cadherin in DU145 cells transfected with microRNA-19a-3p mimic or negative control were detected by Western blot.

RESULTS: Overexpression of microRNA-19a-3p inhibited protein level of SOX4 in DU145 cells. The migration and invasion of DU145 cells were inhibited after transfection of microRNA-19a-3p mimic or negative control in DU145 cells. Protein levels of MMP2, MMP9, N-cadherin, Vimentin, α-SMA and E-cadherin in DU145 cells transfected with microRNA-19a-3p mimic or negative control were detected by Western blot.


Key Words: Prostate cancer, MicroRNA-19a-3p, SOX4.

Introduction

Currently, the incidence of prostate cancer (PCa) ranks the second in the male genitourinary system, just after to bladder cancer throughout the world. The incidence of PCa in the United States is 11/100,000. PCa poses a great challenge in the clinical treatment1-2. The main methods for the early diagnosis of PCa include directorial rectum examination (DRE), prostate cancer specific antigen (PSA) determination and transrectal ultrasound (TRUS)3-4. Tumor stage and Gleason score are the major prognostic factors for PCa5. However, pathological indicators cannot accurately determine the prognosis of PCa, even with the combination of postoperative pathological indicators. Current accuracy in predicting the prognosis of PCa is only 75-85%, which is remarkably lower in those receiving conservative treatment6. Hence, it is of great significance to search for novel prognostic factor of PCa. MicroRNA is a type of single-stranded, non-coding RNA formed by 18-25 nucleotides. MicroRNA inhibits target gene expression by binding to its 3'UTR sequence7. SOX (SRY related high mobility group box) is a kind of transcription factors, and SOX4 belongs to the C group members of SOX family, which was initially found to participate in embryonic development8. The SOX4 gene belongs to a single exon gene and encodes a protein consisting mainly of 474 amino acids located in the nucleus9. Researches10,11 have found that SOX4 is highly expressed in many tumors, especially in PCa. SOX4 exerts a promotive effect on tumors by affecting apoptosis, proliferation and EMT of tumor cells. However, the specific effect of SOX4 on the development and progression of PCa remains unclear. MicroRNA-19a-3p is downregulated in various malignant tumors, such as breast cancer, oral squamous cell carcinoma, and gastric cancer12-14. However, the role of microRNA-19a-3p in PCa is rarely reported. We aim to investigate whether microRNA-19a-3p exerts a regulatory effect on migration, invasion and EMT of PCa cells via targeting SOX4.
Materials and Methods

Cell Culture and Transfection

DU145 cells were cultured in DMEM (Dulbecco’s Modified Eagle Medium) (Gibco, Rockville, MD, USA) containing 10% FBS (fetal bovine serum) (Gibco, Rockville, MD, USA) and maintained in a 5% CO₂ incubator at 37°C. DU145 cells in logarithmic growth phase were inoculated into 6-well plate for overnight culture. MicroRNA-19a-3p mimic or negative control plasmid was transfected into DU145 cells according to the instructions of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). In brief, 8 μL of Lipofectamine 2000 or 100 pmol microRNA-19a-3p mimic were added in 100 μL of OPTI-MEM for 5-min incubation. The two solutions were mixed and maintained at room temperature for 20 min. The transfection mixture was added in the 6-well plate. Culture medium was replaced 4 h later.

RNA Extraction And Quantitative Real-Time-Polymerase Chain Reaction (Qrt-PCR)

TRIzol kit (Invitrogen, Carlsbad, CA, USA) was used to extract the total RNA, which was then reversely transcribed into complementary Deoxyribose Nucleic Acid (cDNA). After the cDNA was amplified, qRT-PCR was performed to detect the expressions of related genes. Primers used in this study were as follows: MicroRNA-19a-3p, F: 5’-CAATCCTCTCAGGCTCAGTCC-3’, R: 5’-TATGCTTGTTCTCGTCTCTGTGTC-3’; U6, F: 5’-CTCGCTTCGGCAGCACA-3’, R: 5’-AACGCTTCAGGAATTTGCGT-3’.

Western Blot

Cells were lysed for protein extraction. The concentration of each protein sample was determined by a BCA (bicinchoninic acid) kit (Abcam, Cambridge, MA, USA). Protein sample was separated by gel electrophoresis and transferred to PVDF (polyvinylidene difluoride) membranes (Millipore, Billerica, MA, USA). After incubation with primary and secondary antibody (Cell Signaling Technology, Danvers, MA, USA), immunoreactive bands were exposed by enhanced chemiluminescence method.

Wound Healing Assay

Transfected DU145 cells were seeded into 6-well plates at a dose of 5×10⁵/mL. When the cell confluence was up to 80%, a sterile 10 μL micropipette tip was used to vertically scratch the cell plate. After removing the exfoliated cells with PBS, serum-free medium was placed for 48 h-incubation. The cell migration was observed under an inverted microscope (Nikon, Tokyo, Japan), and the width of the scratch was measured and photographed.

Transwell Assay

Transfected DU145 cells were centrifuged and resuspended in serum-free Dulbecco’s Modified Eagle Medium (DMEM) at a density of 5.0×10⁴/mL. Transwell chambers pre-coated with Matrigel were placed in 24-well plates. 200 μL of cell suspension and 600 μL of medium containing 10% fetal bovine serum (FBS) were added in the upper and lower chamber, respectively. After cell culture for 48 h, cells were fixed with 4% paraformaldehyde for 15 min and stained with crystal violet for 15 min. Inner cells were carefully cleaned. Penetrating cells were captured in 5 randomly selected fields of each sample.

Statistical Analysis

We used Statistical Product and Service Solutions (SPSS) 16.0 software (IBM, Armonk, NY, USA) for statistical analysis. The quantitative data were represented as mean ± standard deviation (x ± s). The t-test was used for comparing differences between the two groups. p < 0.05 was considered statistically significant.

Results

MicroRNA-19a-3p Mimic Transfection in DU145 Cells

DU145 cells were transfected with microRNA-19a-3p mimic or negative control, respectively, for 48 h. DU145 cells were assigned into mock group (transfection of negative control), control group (no treatment) and microRNA-19a-3p mimic group (transfection of microRNA-19a-3p mimic). QRT-PCR results indicated that microRNA-19a-3p level was remarkably elevated in microRNA-19a-3p mimic group compared with that of mock group and control group. No significant difference in microRNA-19a-3p level was found between mock group and control group (Figure 1A).

Transfection of microRNA-19a-3p mimic in DU145 Cells Inhibited SOX4 expression

Through KEGG analyses, we found that SOX4 was the potential target gene of microRNA-19a-3p
MicroRNA-19a-3p suppresses invasion and metastasis of prostate cancer via inhibiting SOX4

Transfection of microRNA-19a-3p Mimic in DU145 Cells Inhibited Cell Migration

Wound-healing assay was performed to explore the role of microRNA-19a-3p in regulating migration of DU145 cells. The data showed that 24-h and 48-h confluence in control group was 35.63 ± 3.52% and 58.73 ± 4.02%, respectively. In mock group, 24-h and 48-h confluence was 37.23 ± 4.01% and 59.21 ± 4.20%, respectively. Particularly, cell migration was remarkably inhibited in microRNA-19a-3p mimic group, with the 24-h and 48-h confluence of 12.81 ± 1.97% and 23.93 ± 3.08%, respectively (Figure 2A and 2B).

Transfection of microRNA-19a-3p Mimic in DU145 Cells Inhibited Protein Expressions of Matrix Metalloproteinase-2 (MMP2) and Matrix Metalloproteinase-9 (MMP9)

Transfection of microRNA-19a-3p mimic in DU145 cells inhibited protein expressions of MMP2 and MMP9. However, we did not observe remarkable differences in protein expressions of MMP2 and MMP9 between mock group and control group (Figure 4A and 4B).

Transfection of MicroRNA-19a-3p Mimic in DU145 Cells Inhibited Protein Expressions of EMT-Related Genes

Transfection of microRNA-19a-3p mimic in DU145 cells downregulated protein expressions of EMT-related genes.
Figure 2. MicroRNA-19a-3p mimic transfection inhibited invasion of DU145 cells. A, Confluence degree in mock group, control group and microRNA-19a-3p mimic group at 0, 24 and 48 h (error bar=50 μm). B, Analyses of cell confluence in mock group, control group and microRNA-19a-3p mimic group.

Figure 3. MicroRNA-19a-3p mimic transfection inhibited migration of DU145 cells. A, Invasive cells in mock group, control group and microRNA-19a-3p mimic group at 0, 24 and 48 h (error bar=20 μm). B, Analyses of cell invasion in mock group, control group and microRNA-19a-3p mimic group.
MicroRNA-19a-3p suppresses invasion and metastasis of prostate cancer via inhibiting SOX4

Discussion

PCa is one of the common malignancies in males. Globally, about 110,000 patients die of PCa every year. Metastatic PCa is frequently seen, seriously affecting the long-term survival of PCa patients\textsuperscript{15,16}. With the advanced progress of molecular researches, gene therapy exerts promising perspective in tumor treatments. Recent works confirmed that some certain microRNAs are differentially expressed in PCa, regulating the proliferation of tumor cells and tumor growth. Meanwhile, microRNAs that are related to metastasis and invasion of malignant tumors have been well concerned\textsuperscript{17,18}. In this study, the specific role of microRNA-19a-3p in migration and invasion of DU145 cells was detected after transfection of microRNA-19a-3p mimic. Overexpression of microRNA-19a-3p remarkably decreased migratory and invasive capacities of DU145 cells. Besides, microRNA-19a-3p overexpression downregulated MMP2, MMP9, N-cadherin, Vimentin, α-SMA, but upregulated E-cadherin, suggesting that mi-
microRNA-19a-3p could inhibit EMT of PCa cells. Our results confirmed that microRNA-19a-3p is involved in the migration, invasion and EMT of PCa. The clinical importance of SOX4 has been well recognized. Numerous studies have shown that SOX4 may promote the occurrence and progression of tumors. For example, SOX4 is overexpressed in triple-negative breast cancer. Further analyses showed that as a prognostic factor, SOX4 promotes the progression of triple-negative breast cancer via regulating EMT\(^9,20\). SOX4 is also highly expressed in colon cancer, gastric cancer, melanoma, pancreatic cancer, prostate cancer, and lung cancer\(^10,21,22\). Overexpressed SOX4 in liver cancer cells promoted metastasis via EMT\(^23\). Additionally, SOX acts as an oncogene in acute lymphoblastic leukemia and acute myeloid leukemia\(^24\). Wang et al\(^10\) have found that overexpression of SOX4 can promote the metastasis of PCa by regulating EMT. SOX4 knockdown in invasive PCa cells greatly inhibits tumor metastasis of PCa by regulating EMT. SOX4 knockdown significantly inhibited the migration and invasion of PCa cells in vitro. These results indicate that SOX4 promotes the progression of PCa.

Conclusions

We found that microRNA-19a-3p inhibits migration, invasion and EMT of PCa cells via inhibiting SOX4, which provides novel directions for developing target therapy of PCa.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References


5) Liu X, Tang J, Fei X, Li QY. Prostate-specific antigen (PSA) density and free to total PSA ratio in diagnosing prostate cancer with prostate-specific antigen levels of 4.0 ng/ml or less. Iran J Public Health 2015; 44: 1466-1472.


MicroRNA-19a-3p suppresses invasion and metastasis of prostate cancer via inhibiting SOX4


