Original Article
Effect of miR-188-5p on growth and metastasis of prostate cancer cells

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Abstract: Objective: To investigate the role of miR-188-5p in proliferation, migration, and invasion of prostate cancer cell lines LNCaP, and the possible mechanism. Methods: The expression of miR-188-5p in prostate cancer cell lines LNCaP and prostate cancer tissues were detected by qPCR; miR-188-5p mimics was transfected into LNCaP cells using Lipo2000 solutions. The effects of miR-188-5p mimics on the proliferation, migration, and invasion were detected by CCK-8 assay, Transwell assay. The expression level of phosphatase and tensin homologue (PTEN) in LNCaP cells influenced by miR-188-5p mimics was detected by Western blot. Results: qPCR results manifested that the expression levels of miR-188-5p in LNCaP cells or prostate cancer tissues were significantly higher than that in control group (P<0.001). MiR-188-5p overexpression could obviously promote the proliferation, migration and invasion ability of LNCaP cells, compared with those in miRNA control group (P<0.001). And miR-188-5p overexpression could down-regulate the expression of PTEN. Conclusions: miR-188-5p overexpression could remarkably promote proliferation, migration, invasion ability of prostate cancer cells, which may be associated with down-regulation of PTEN.

Keywords: miR-188-5p, proliferation, migration, invasion, prostate cancer

Introduction
Prostate cancer is one of the most commonly diagnosed cancers among the elderly men. It has become the second main cause of male cancer-related mortality [1, 2]. In recent years, the incidence and mortality rate of prostate cancer have increased remarkably according to the epidemiological survey [3]. Although significant progress has been made in treatment of prostate cancer, the prognosis of patients with prostate cancer is still poor due to high metastasis [4]. Therefore, it is necessary to investigate the molecular mechanism underlying development of prostate tumors, which enable us to identify novel therapeutic targets and prognostic markers.

MiRNAs, as small, noncoding RNA molecules have been reported to participate in the cell activity and biological processes via regulating encoded proteins expression [5]. Increased researches showed that there was a relationship between miRNAs expression and prostate cancer development [6, 7]. Some specific miRNAs have been confirmed in regulation of prostate cancer cells activities such as proliferation, metastasis and differentiation, suggesting miRNAs could be considered as a specific markers and benefit to the clinical therapy and prognosis of cancer [8]. It was reported that dysregulation of miR-188-5p expression existed in liver cancer and oral squamous cell carcinoma [9, 10]. Wang et al reported that miR-188-5p promoted the proliferation and migration of gastric cancer cells through transcriptional up-regulation of Sal-like protein 4 [11]. However, the detailed function and mechanism of miR-188-5p in the development of prostate cancer is still unclear.

Phosphatase and tensin homologue (PTEN), as tumor suppressor, is composed of 9 exogenous factors and 8 inclusive factors and could phosphatase activity against proteins [12]. Previous studies that PTEN could significantly inhibit the growth, metastasis and invasion of tumor cells [13]. Compared with normal people, the levels of PTEN is remarkably lower in the occurrence and development of prostate cancer [14].
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mechanism experiments related with prostate cancer demonstrated that PTEN is the target mRNA of miR-410-3p which exerts oncogenic functions [15]. As we can see, PTEN is a potential treatment target for prostate cancer.

In this study, we detected the expression level of miR-188-5p in prostate cancer tissues and LNCaP cells, and the role of miR-188-5p in proliferation, migration and invasion of LNCaP cells. Moreover, we also explored the possible mechanisms. This research would provide new target for therapy of prostate cancer.

Materials and methods

Clinical specimens

From February 2019 to June 2020, a total of 20 pairs of prostate tumor and adjacent non-tumorous prostate tissues were randomly selected from patients underwent prostate operation without any chemotherapy or radiotherapy in our hospital. The specimens were snap-frozen in liquid nitrogen and kept at -80°C for later RNA or protein extraction. All of the cases were confirmed as prostate through pathological diagnosis. All human specimens were obtained with informed consent and this study was approved by the hospital ethic committee.

Cell culture and transfection

Human prostate cancer cell lines LNCaP and normal prostatic epithelial cell lines RWPE-1 were cultured in Dulbecco's modified eagle medium (DMEM) (Gibco, USA) containing 10% fetal bovine serum (FBS) (Gibco, USA) and 100 μg/mL streptomycin and 100 μg/mL penicillin. All the cells were kept in an incubator with the conditions of 5% CO₂ and 37°C. Cells in logarithmic growth phase were transfected with miR-188-5p mimics and control vector using lipofectamine 2000 Reagent (Invitrogen, USA) according to the manufacturer's instructions.

CCK-8 assay

The detection of cell proliferation was performed by CCK8 (Dojindo, Japan) assay. After transfection with miR-188-5p mimics or control vector, 200 μL LNCaP cells were added into a 96-well plate by the density of 2,000 cells per well. All the cells were cultured in an incubator with the condition of 5% CO₂ and 37°C. 10 μL CCK8 solutions were added into each well at 24 h, 48 h and 72 h after culture. The cells were continuing to be incubated for another 4 h. The absorbance of each well at 450 nm was detected with microplate reader (Bio-Tek Instruments, USA).

Cell migration and invasion assay

LNCaP cells were transfected with miR-188-5p mimics or control vector and incubated to near confluence (>70) in 24-well dishes. After digesting cells with trypsin, LNCaP cells were added into Transwell chambers. Fetal bovine serum was put into the lower chamber as a chemoattractant. After culturing for 24 h in a incubator, migrated cells located on the lower surface of the Transwell chambers were stained using 0.1% crystal violet liquid (Sigma, USA). And unmigrated cells on the upper surface of the Transwell chambers were removed with cotton swap. Then, the crystal violet was eluted completely using 33% acetic acid dehydrating, and the optical density (OD) of the elution liquid at 570 nm was measured using microplate reader (Bio-Tek Instruments, USA). OD values indicated the migration ability of LNCaP cells.

Matrigel was diluted with DMEM solution and spread evenly on the membranella (8 μm) of Transwell. The subsequent procedure was conducted according to the method of cell migration assay. Invasion assay were assessed by the ability of cells passing through Matrigel-coated membrane matrix and OD values indicated the invasion ability of LNCaP cells.

Real-time PCR analysis

According to the manufacturer’s instructions, Total RNA of prostate cancer tissues and prostate cancer cell lines LNCaP were extracted using Trizol Reagent (Invitrogen, USA). Using TaqMan MicroRNA reverse transcription Kit (Promega, USA), the above extraction of RNA was synthesized into cDNA. Internal reference U6 was used for normalization. And Real-time PCR was conducted using SYBRH Premix Ex TaqTM (TaKaRa, Japan). The primers were designed as follows, miR-188-5p upstream primer: 5’-ACACTCCAGCTGGCGAC3’, downstream primer: CCAGTGCAGGG TCCGAGGT-3’. PCR am-
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plification was conducted according to the following conditions: predenaturisation 5 min in 94°C, denaturation 20 s in 94°C and annealing/extension 1 min at 60°C, a total of 40 cycles. The relative expression amount of targeted gene was calculated by 2-ΔΔCt method using ABI 7300 System software.

Western blot analysis

After transfection with miR-188-5p or control vector, Total proteins of cells were extracted using protein lysis buffer RIPA. The concentration of proteins in each group was detected using BCA method. Proteins were isolated through SDS-PAGE gel electrophoresis and subsequently transferred onto PVDF membrane. Then the membrane was incubated in the TBST of 5% non-fat milk powder and blocked for 1 h at room temperature. The antibody against PTEN (Santa Cruz, USA) (dilution: 1:1000) was incubated with membranes overnight in the shaker at 4°C. After wash, PVDF membrane was incubated with HRP-conjugated secondary antibody (dilution: 1:500) for 1 h. ECL reagent was used to assess the expression of proteins. Bio-Rad image software was used to quantify the target band intensities. GAPDH served as an internal control.

Statistical methods

SPSS 22.0 software was used to conduct the statistical analysis in this study. The measurement data are presented as mean ± standard deviation (SD). And the comparison between two groups was conducted by T test. Enumeration data was expressed as cases or percentage and the comparison between two groups was performed using χ² test. P<0.05 was considered as significantly statistical differences [16].

Results

The expression of miR-188-5p in prostate cancer tissue

Expression levels of miR-188-5p in prostate cancer tissue were examined by qPCR. The results showed that in contrast to normal adjacent tissues, the expression levels of miR-188-5p were obviously higher in prostate cancer tissue. And there were significantly statistical differences between two groups (1.06±0.27 vs 3.12±0.54, P<0.001), as shown in Figure 1.

The expression of miR-188-5p in prostate cancer cells LNCaP

According to qPCR results, compared with normal prostatic epithelial cell lines RWPE-1, the expression of miR-188-5p was much higher in prostate cancer cells LNCaP, and The significantly statistical differences could be found (1.02±0.19 vs 3.12±0.54, P<0.001), as seen in Figure 2.

Effects of miR-188-5p mimics on the proliferation of LNCaP cells

CCK-8 experiment showed that the OD values in miR-188-5p mimics group at 24 h, 48 h and 72 h after culture was obviously higher than those in miRNA control group, and the significant statistical difference was found (all P<0.05), as shown in Table 1.

Figure 1. Comparison of miR-188-5p expression level between prostate cancer and adjacent cancer. Compared with adjacent cancer, ***P<0.001.

Figure 2. Comparison of miR-188-5p expression level between LNCaP cells and RWPE-1 cells. Compared with RWPE-1 cells, ***P<0.001.
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Table 1. Comparison of OD values between miR-188-5p mimics group and miRNA control group

<table>
<thead>
<tr>
<th>Groups</th>
<th>OD values</th>
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<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>24 h</td>
<td>48 h</td>
<td>72 h</td>
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<td>miRNA control group</td>
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<td>miR-188-5p mimics group</td>
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<tr>
<td>P value</td>
<td>0.505</td>
<td>0.028</td>
<td>0.005</td>
<td>0.002</td>
</tr>
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</table>

Effects of miR-188-5p mimics on the migration of LNCaP cells

At 24 h after LNCaP cells transfected with miR-188-5p mimics, compared with miRNA control group (OD value: 0.59±0.10), miR-188-5p mimics remarkably promoted the migration ability of LNCaP cells (OD value: 1.08±0.14), as shown in Figure 3. And there were significantly statistical differences between two groups (P<0.001).

Effects of miR-188-5p mimics on the invasion of LNCaP cells

When the expression level of miR-188-5p was up-regulated, the invasion ability of LNCaP cells was obviously improved (OD value: 0.97±0.12). Compared with miRNA control group (OD value: 0.43±0.09), there was a significant difference with P<0.001, as shown in Figure 4.

Effects of miR-188-5p mimics on the expression of PTEN in prostate cancer cells LNCaP

As shown in Figure 5, miR-188-5p mimics remarkably increased the expression of PTEN in prostate cancer cells LNCaP, compared with that in miRNA control group (P<0.001).

Discussion

Prostate cancer is one of the most common human malignancies worldwide, and seriously threatens physical and psychological health in patients [17]. Due to the highly malignant potential, the long-time survival of patients with prostate cancer is unsatisfactory. The development of prostate cancer involves lots of oncogenes and tumor suppressor genes [18]. And a better understanding of molecular mechanism underlying the malignancies of prostate cancer is very crucial for the diagnosis, prevention and therapy of this disease.

Increase evidences demonstrated an important role of miRNAs in human cancer [19]. The expression profiling of miRNAs was usually used for classification of tumor stages and prognosis and miRNAs could influence gene silencing via involving in lots of complex gene regulatory networks [20]. The discovery of miRNAs could provide new ideas for prostate cancer treatment. Several types of miRNA such as miR-410-3p, miR-421, and miR-338-5p and
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miR-345-5p have been reported to play key roles in development of prostate cancer [21, 22]. MiR-188-5p is a newly found member of miRNA family and down-regulation of miR-188-5p was confirmed in homocysteine-induced cardiac remodeling and synaptic transmission [23, 24]. In term of cancer researches, current studies showed that miR-188-5p was significantly low expression in bladder cancer and gastric cancer [25]. Yang et al reported that miR-188-5p could promote epithelial-mesenchymal transition via targeting DNA binding 4 through Wnt/β-catenin signaling in development of retinoblastoma [26].

In this study, it was found that the overexpression level of miR-188-5p was contributed to the carcinogenesis activities. qPCR results showed that the expression level of miR-188-5p in prostate cancer tissues was significantly increased compared with adjacent cancer tissues. In prostate cancer cells LNCaP, this research also showed the expression level of miR-188-5p was highly expressed compared with normal epithelial cell lines RWPE-1. The role of miR-188-5p up-regulation in proliferation, migration and invasion of LNCaP were detected by CCK-8 and Transwell assays. It was demonstrated that miR-188-5p overexpression could obviously promote the proliferation, migration and invasion of prostate cancer cells, suggesting that miR-188-5p served as a carcinogenic effector in prostate cancer. The above results were consistent with the effect of miR-188-5p in other kinds of tumors reported previously [27]. As we can see, miR-188-5p could promote the growth of prostate cancer by inducing cells proliferation. miR-188-5p

**Figure 4.** Effects of miR-188-5p mimics on the invasion ability of LNCaP cells. A: OD value of invasive cells; B: Crystal violet staining; Compared with miRNA control group, ***P<0.001.

**Figure 5.** Effects of miR-188-5p mimics on the expression of PTEN in LNCaP cells. A: Western blot of PTEN; B: Relative expression of PTEN protein; Compared with miRNA control group, ***P<0.001.
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could promote the migration of prostate cancer cells, which was very crucial process of neoplasm metastasis. miR-188-5p could promote invasion of the prostate cancer cells, which was associated with cell migration and neoplasm metastasis. Therefore, these data have indicated that miR-188-5p could be a novel target for prostate cancer.

This study also demonstrated that miR-188-5p inhibited the expression of PTEN protein in prostate cancer cells by Western blot method. The function of PTEN was known to be control via various mechanisms at the levels of transcription, post-transcription and post-translation. PTEN could regulate an array of physio-pathological processes associated with proliferation, invasiveness, apoptosis and differentiation. Some studies reported that there was loss of PTEN in approximate 68% and 44% of primary prostate cancer [28]. Animal studies showed that full genetic deletion of PTEN in developed prostate gland could lead to rapid high grade prostate intraepithelial neoplasia, and even invasive prostate cancer [29]. The effect of PTEN as a tumor suppressor of prostate cancer is well established [30]. In the present study, we found that miR-188-5p mimics could obviously down-regulated the expression of PTEN in prostate cancer cells lines LNCaP. The above results indicate that miR-188-5p may affect the proliferation, migration and invasion of prostate cancer cells through the decreased expression of PTEN protein in processes of prostate cancer.

In conclusion, this study demonstrated that miR-188-5p was up-regulated in prostate cancer cells LNCaP and prostate cancer tissues, and miR-188-5p overexpression could remarkably promote proliferation, migration and invasion of LNCaP cells, which may associated with down-regulation of PTEN protein. Our findings indicated that miR-188-5p could be a potential target for molecular-targeted therapy of prostate cancer. However, it is needed to make furtherly investigation of miR-188-5p-regulated target genes and involved signaling transduction pathways, so as to provide new ideas for the treatment of prostate cancer.

Disclosure of conflict of interest
None.

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