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Inhibition of miR-720 suppresses cell migration and invasion in prostate cancer by targeting StarD13.

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Abstract

StAR related lipid transfer domain containing 13 (StarD13) plays an important role in the development and progress of multiple cancers. However, the effects of StarD13 in prostate cancer remain unclear. This study aims to investigate the effects of microRNA-720 (miR-720) inhibition in prostate cancer progress by targeting StarD13. The results of this research showed that miR-720 was highly expressed in prostate cancer cells. The inhibition of miR-720 repressed proliferation, migration and invasion in prostate cancer cells. StarD13 was predicted as a target gene of miR-720 by bioinformatics analysis. qRT-PCR, Western bolt analysis and dual-luciferase reporter assay were performed to confirm the prediction. Taken together, our results suggested that miR-720 plays an important role in cell proliferation, migration and invasion of prostate cancer by targeting StarD13.

Keywords: microRNA-720, Prostate cancer, StarD13, DU145, Migration, Invasion.

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Introduction

Prostate cancer is one of the most common cancers in men and its cancer death is only behind lung and bronchus cancer in the United States [1]. There are significant differences in the incidence and mortality rates for prostate cancer between Western countries and Asian, higher rates in Western countries and lower rates in Asian [2]. However, in recent years, the morbidity and mortality of prostate cancer in Asia have been increasing continuously, and the rate of growth is more rapid than that of developed countries [3]. Therefore, it is extremely urgent to delve novel targets that regulate the progress of prostate cancer.

microRNAs (miRNAs) are a class of 22-noncoding nucleotides encoded by endogenous gene, which regulate gene expression through binding to the 3'-untranslated region (UTR) of target mRNAs. Recent studies showed that miRNAs can be used as diagnostic and prognostic biomarkers of prostate cancer [4], including miR-1271 [5], miR-1297 [6], miR-126 and 149 [7]. They have been implicated in the regulation of cell proliferation, migration and invasion in prostate cancer.

In this study, we explored the role of miR-720 in prostate cancer progress. Bioinformatics analysis predicted that StarD13 is a target gene of miR-720. Therefore, we transfected DU145 cells with miR-720 inhibitor and evaluated the effects on the abilities of cell proliferation, migration and invasion in DU145 cells by targeting StarD13.

Materials and Methods

Cell culture and transfection

Human normal prostate epithelial cell line RWPE-1 was purchased from Cell Bank of Chinese Academy of Sciences (Shanghai, China) and cultured in Keratinocyte-SFM (K-SFM) supplemented with 10% fetal bovine serum. Human prostate cancer cell line DU145 was obtained from American Type Culture Collection (ATCC) and cultured in Eagle's Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum. The cells were maintained in 5% CO_2 atmosphere at 37°C.

DU145 cells were transfected with the empty vector (negative control, NC) or miR-720 inhibitor by Lipofectamine 2000 (Invitrogen, USA), according to the manufacture's protocol.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from cells using Trizol Reagents (Invitrogen, USA) and then was Reverse Transcribed (RT) into complementary DNA (cDNA) by PrimeScript™ RT reagent Kit (Perfect Real Time) (Takara Biotechnology Co., Ltd, Dalian, China). The cDNA was quantified by qRT-PCR using SYBR® Green master mix kit (Takara Biotechnology Co., Ltd, Dalian, China). The expression of miR-720 mRNA was normalized to U6, and the expression of StarD13 was normalized to GAPDH. The data were analysed using the $2^{-\Delta\Delta Ct}$ method. The following primers were used: StarD13, forward 5'

CGAGGAGACAGAAATGGGTCA 3', reverse 5' TCCACTGCTTTCGCTGTGAAT 3'; GAPDH, forward 5' GGAGCGAGATCCCTCCAAAAT 3', reverse 5' GGCTGTTGTCATACTTCTCATGG 3'.

CCK8 assay

DU145 cells were incubated into 96-well plates and cultured for 24 h. Then cells were no treated (Control) or treated with the empty vector (negative control, NC) or miR-720 inhibitor for 12 h, 24 h, 48 h, 72 h. CCK8 was added into each well and incubated at 37°C for 2 h. The absorbance was measured at 450 nm using a microplate reader (Bio-Rad 680).

Wound healing assay

DU145 cells were inoculated into 6-well plates and cultured for 24 h. The monolayer cells were scratched with pipette tips. Then cells were no treated (Control) or treated with the empty vector (negative control, NC) or miR-720 inhibitor for 24 h. The wound was recorded by a microscope.

Cell invasion assay

The invasion ability of DU145 cells in different groups was assessed using Matrigel-coated transwell chamber (BD Bioscience, CA, USA). Cells were seeded into the upper chamber. After incubation for 24 h, cells in the lower chamber were fixed with 4% paraformaldehyde, stained with 0.1% crystal violet, and counted under a microscope.

Western blot analysis

After treatment, DU145 cells in different groups were harvested and lysed in ice-cold RIPA lysis buffer (20 mM Tris-HCl PH 7.4, 1 mM EDTA, 150 mM NaCl, 1% (v/v) Triton X-100, 0.5% sodium deoxycholic acid) for 30 min. Protein concentrations were measured by a NanoDrop instrument. Equal amounts of proteins (20 μg) were separated by 6-10% SDS-PAGE (Beyotime and Biotechnology, Shanghai, China) and transferred to Polyvinylidene Difluoride (PVDF, Millipore, USA) membranes. Then membranes were blocked with dried skimmed milk for 1 h at room temperature, incubated with the primary antibodies (StarD13, sc-377054, 1:1000, Santa Cruz Biotechnology, Inc., USA; GAPDH, sc-32233, 1:1000, Santa Cruz Biotechnology, Inc., USA) overnight at 4°C, and incubated with HRP-labeled Goat Anti-Mouse IgG (A0216, 1:1000, Beyotime and Biotechnology, Shanghai, China) for 2 h at room temperature. Finally, the bands were detected with BeyoECL Plus (P0018, Beyotime and Biotechnology, Shanghai, China).

Dual-luciferase reporter assay

The binding sites for miR-720 with the 3'-UTR of StarD13 were predicted by TargetScan 7.1 (http://www.targetscan.org). Then dual-luciferase reporter assay was performed using Dual Luciferase Reporter Gene Assay Kit (RG027, Beyotime and Biotechnology, Shanghai, China) according to the manufacturer's instructions after transfection.

Statistical analysis

All results were presented as mean ± SD. Statistical analyse was carried out using SPSS software version 21.0 (SPSS). Differences were analysed using Student's t-test. A P value of less than 0.05 was considered significant.

Results

miR-720 is highly expressed in prostate cancer cells

The expression of miR-720 in RWPE-1 and DU145 cells was tested by qRT-PCR. As shown in Figure 1A, miR-720 is higher expressed in prostate cancer cells than normal prostate epithelial cell. These results suggested that miR-720 may be an oncogene in prostate cancer.

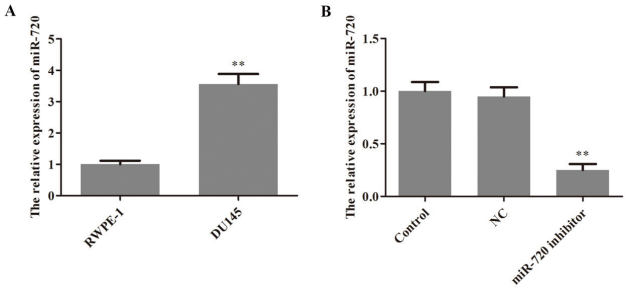

Figure 1. *The expression of miR-720 in cells. (A) The relative expression of miR-720 in RWPE-1 and DU145 cells was detected by qRT-PCR; $^{**}P<0.01$. (B): Transfection of miR-720 inhibitor significantly decreased the expression level of miR-720 in DU145 cells; $^{**}P<0.01$.*

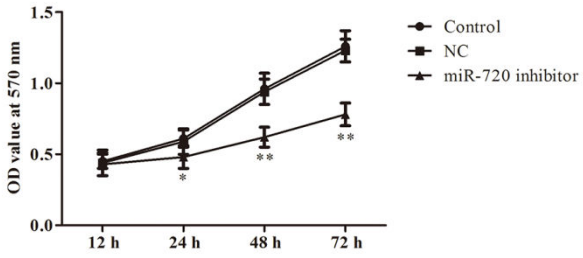

Figure 2. *Down-regulated miR-720 expression inhibits cell proliferation. DU145 cells were transfected with miR-720 inhibitor for 12, 24, 48 and 72 h and cell proliferation were measured by CCK8 assay. $^{*}P<0.05$, $^{**}P<0.01$.*

miR-720 inhibitor suppresses cell proliferation, migration and invasion

To carry out the function of miR-720 in prostate cancer, DU145 cells were cultured and transfected with miR-720 inhibitor. Compared with Control group and NC group, the expression level of miR-720 was significantly down-regulated (Figure 1B). CCK8 assay showed that down-regulated miR-720 expression markedly inhibited the proliferation of DU145 cells (Figure 2). Wound healing assay showed that down-regulated miR-720 expression significantly suppressed the migration of DU145 cells (Figure 3). The Transwell

invasion assay showed that down-regulated miR-720 expression observably attenuated the invasion of DU145 cells (Figure 4). Taken together, these findings suggested that miR-720 inhibitor suppresses cell proliferation, migration and invasion.

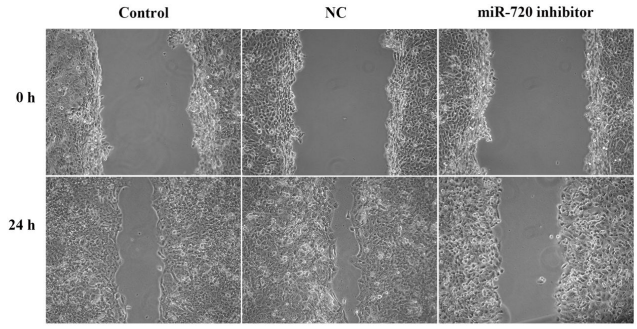

Figure 3. *Down-regulated miR-720 expression inhibits cell migration. DU145 cells were transfected with miR-720 inhibitor for 24 h and cell migration were detected by wound healing assay.*

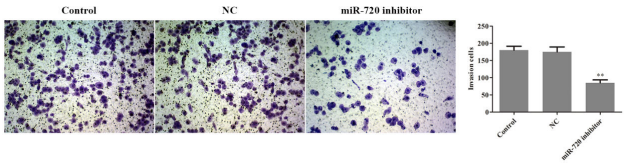

Figure 4. *Down-regulated miR-720 expression inhibits cell invasion. DU145 cells were transfected with miR-720 inhibitor. Cell invasion were detected by Transwell assay.* $^{**}P<0.01$.

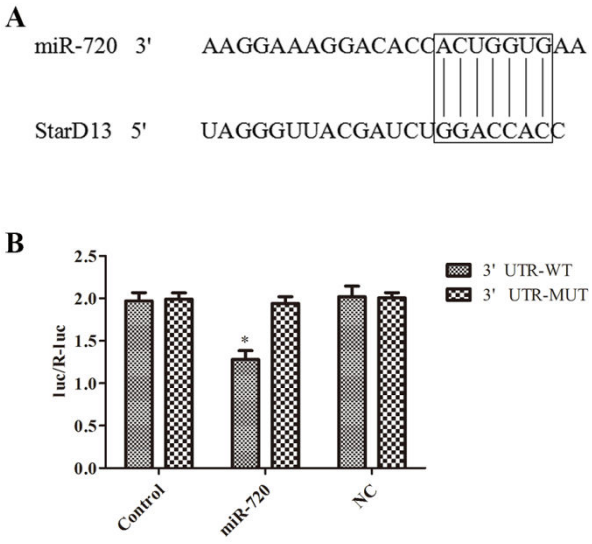

Figure 5. *StarD13 is a direct target of miR-720 in DU145 cells. (A) The 3'-UTR of StarD13 mRNA was found to contain the complementary sequence of miR-720. (B) Down-regulated miR-720 expression significantly suppressed the dual-luciferase activity of WT 3'-UTR of StarD13 but not MUT 3'-UTR of StarD13;* $^{*}P<0.05$.

StarD13 is a direct target of miR-720 in DU145 cells

We predicted that miR-720 could target the 3'-UTR of StarD13 by bioinformatics analysis. Dual-luciferase reporter assay was performed to confirm whether miR-720 can bind to the 3'-UTR of StarD13 (Figure 5A). The results showed that miR-720 inhibitor decreased the luciferase activity of wild-type (WT) StarD13 3'-UTR (Figure 5B). To further verify that StarD13 is a direct target of miR-720, the expression levels of StarD13 mRNA and protein were measured by qRT-PCR and western blot. As shown in Figure 6, miR-720 inhibitor increased the levels of StarD13 mRNA (Figure 6A) and protein (Figure 6B). Overall, these results suggested that StarD13 is a direct target of miR-720.

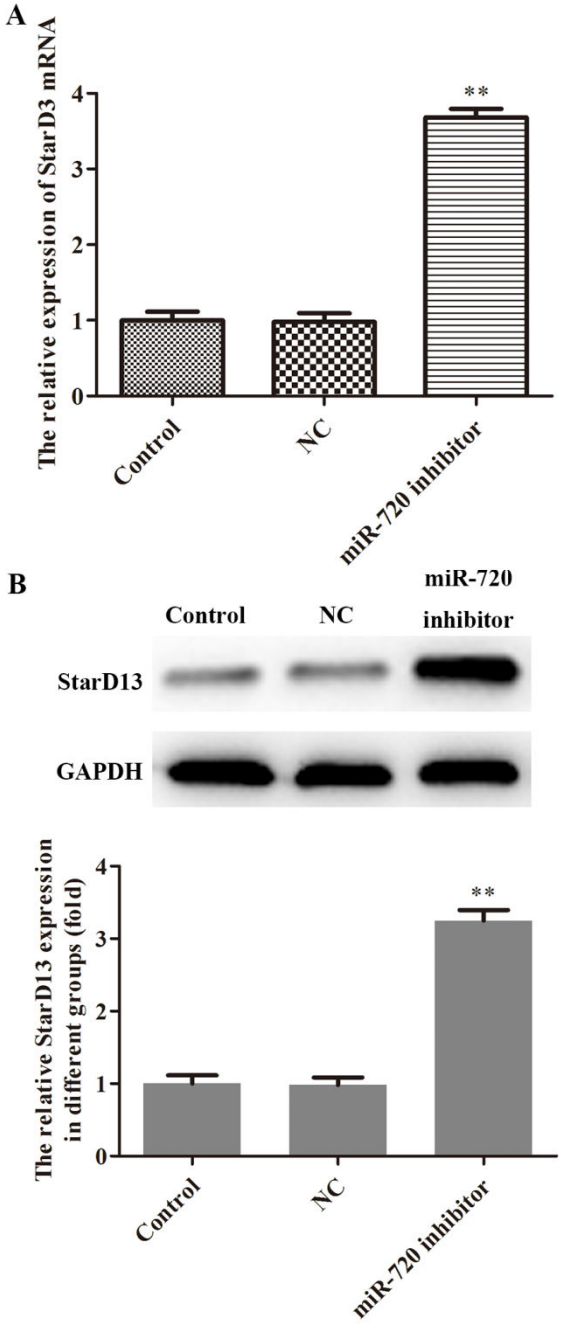

Figure 6. *Down-regulated miR-720 expression promotes the expression of StarD13. DU145 cells were transfected with miR-720 inhibitor. (A) The expression of StarD13 mRNA was assessed by qRT-PCR;* $^{**}P<0.01$. *(B) The expression of StarD13 protein was measured by western blot analysis;* $^{**}P<0.01$.

Discussion

The aging population and economic development in Asia speed up the change of lifestyle to Westerners, and prostate cancer may gradually become a more serious medical and social economic problem [3]. miRNAs play a key role in the progress of many cancers. It has been reported that miR-720 can regulate the progress of breast cancer [8], triple-negative breast cancer [9] and colorectal cancer [10]. However, the role that miR-720 plays in prostate cancer remains unknown. In this study, we found that miR-720 was highly expressed in prostate cancer DU145 cells by qRT-PCR. The data indicated that miR-720 may be an oncogene of prostate cancer.

To further explore the role of miR-720 in prostate cancer, DU145 cells were transfected with miR-720 inhibitor. Then, the results of CCK8 assay showed that downregulation of miR-720 repressed cell proliferation of DU145 cells.

Tumor metastasis is a leading cause of death in prostate cancer patients. Therefore, we performed wound healing and Transwell assays to detect the ability of migration and invasion in DU145 cells. Silencing of miR-720 suppressed prostate cancer DU145 cells migration and invasion. These results further verified that miR-720 may play an important role in the progress of prostate cancer.

StAR related lipid transfer domain containing 13 (StarD13), also known as deleted in liver cancer 2 protein (DLC-2), may be involved in regulation of cell proliferation and cell apoptosis, acting as a tumor suppressor in hepatocellular carcinoma [11,12]. In Chang's study, StarD13 was proved to be a direct and functional target of miR-125b in gastric cancer, promoting invasion and metastasis [13]. In the current study, StarD13 was predicted as a direct target of miR-720 in prostate cancer by bioinformatics analysis. Then, this prediction was validated by dual-luciferase reporter assay. Furthermore, silencing of miR-720 up-regulate the expression of StarD13 mRNA and protein.

Taken together, our data suggested that miR-720 plays an important role in cell proliferation, migration and invasion of prostate cancer DU145 cells, promoting the progress of prostate cancer. Meanwhile, StarD13 is proved to be a direct and functional target of miR-720 in prostate cancer.

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