Biomedical Research 2017; 28 (22): 9813-9816

ISSN 0970-938X
www.biomedres.info

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.

Accepted on October 11, 2017

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-nucleotides 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'