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Original article

Small interference RNA-mediated silencing of prostate stem cell antigen attenuates growth, reduces migration and invasion of human prostate cancer PC-3M cells☆

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Abstract

Objectives: Prostate stem cell antigen (PSCA), a glycosylphosphatidylinositol (GPI)-anchored cell surface glycoprotein, is highly expressed in both local and metastatic prostate cancer (CaP). Elevated PSCA expression has been shown to correlate with malignant phenotype and clinical progression. The purpose of the current study is to investigate the therapeutic potential of small interference RNA (siRNA) targeting PSCA on human CaP cells.

Materials and methods: A set of two siRNAs directed different regions of human PSCA (siRNA-PSCA) were designed and transfected into a human CaP PC-3M cell line. The silencing effect was screened by RT-PCR and Western blotting. The biological effects of siRNA-PSCA on PC-3M cells were investigated by examining the cell proliferation through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, cell cycle distribution through flow cytometry, and migration and invasion potencies through transwell invasion assay upon the PSCA silencing.

Results: PC-3M cells had positive PSCA expression on immunocytochemical assay. PSCA expression was depleted at 48 hours after transfection with siRNA-PSCA. Silencing of PSCA significantly suppressed cell proliferation. Cell cycle assay showed that the anti-proliferation effect of siRNA-PSCA was mediated by arresting cells in the G_0/G_1 phase rather than apoptosis. Furthermore, PSCA knockdown resulted in a marked decrease of cell migration and invasion capabilities in PC-3M cells.

Conclusions: The present study provides the first evidence that silencing PSCA using siRNA can inhibit the proliferation and invasiveness properties of human CaP cells, which may provide a promising therapeutic strategy for CaP and open a novel avenue toward the investigation of the role of PSCA overexpression in cancers.

Keywords: Prostatic adenocarcinoma (CaP); Prostate stem cell antigen (PSCA); RNA interference (RNAi)

1. Introduction

Prostate cancer (CaP) has emerged as the most commonly diagnosed malignancy and the second leading cause of cancer-related death in men in the Western countries. Although there have been advances in the management of primary, localized CaP with definitive treatment with the intent to cure (i.e., radical prostatectomy and/or radiation therapy), a substantial number of those patients will suffer biochemical recurrence and eventually progress to metastatic disease. Unfortunately, the only therapeutic options presently available for metastatic, hormone-refractory CaP or for those who ultimately fail local treatment are temporizing or extend life only modestly. Therefore, novel therapeutic approaches and strategies to combat CaP are urgently needed. Differences in gene expression between normal cells and cancer cells often provide interesting targets for anti-neoplastic therapy. For this reason, considerable efforts have been made to understand the genetic controls of cellular proliferation and cell division clearly, which may provide the basis for the rational design of therapeutic strategies for the management of CaP.

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Prostate stem cell antigen (PSCA) is a glycosylphosphatidylinositol (GPI)-anchored 123 amino acid cell surface glycoprotein that belongs to the Thy-1/Ly-6 family, and is predominantly CaP-specific [1]. In situ hybridization and immunohistochemical analysis demonstrated that PSCA is expressed by a large proportion of human prostate tumors, including high-grade prostatic intraepithelial neoplasia, primary androgen-dependent cancers, hormone-refractory metastases, but has restrictive expression in normal tissues [1–5]. Elevated PSCA expression levels in prostatic carcinoma are shown to significantly correlate with increased Gleason grade and tumor stage, progression to androgen independence, prostatic capsular invasion, seminal vesicle involvement, and biochemical recurrence and/or distant metastases [1–4]. PSCA expression is also particularly increased in bone metastatic lesions examined. All these data support the role of PSCA in CaP biology and make PSCA a compelling therapeutic target. Different immunotherapy approaches against PSCA have been tested in preclinical models, including cancer vaccine, therapeutic monoclonal antibodies, and antibody conjugated to toxic drugs [6–11]. Based on those various experimental and clinical research findings, blocking the PSCA expression would be considered as a rational strategy to manage this disease. However, the biological effects of deleting endogenous PSCA expression on human CaP cells have not been investigated yet. In addition, recent reports have shown that PSCA expression was also up-regulated in other few nonprostatic malignancies, including superficial transitional cell carcinoma of the bladder [12], clear cell renal cell carcinoma [13], and pancreatic cancers [14]. However, the precise role and function of PSCA in various human malignancies are largely unknown.

Up to now, although chemical inhibitors of the oncogene expression have been used to induce growth disadvantage to cancer cells, they have the intrinsic disadvantage that they often evoke nonspecific side effects. Sequence-based approaches such as conventional antisense technologies provide an attractive alternative but usually offer only a transient and partial suppression of the gene of interest. The development of 21-nucleotide siRNAs specifically recognizing particular mRNA sequences provides new powerful reagents to selectively down-regulate gene expression and holds great potential not only for the development of gene-specific therapeutic agents but also for the analysis of gene function [15]. In the present study, the exogenously synthetic 21-nucleotide siRNA molecularly targeting human PSCA gene was transfected into a highly malignant human CaP cell line PC-3M. We intend to evaluate the therapeutic potential of silencing the PSCA expression with siRNA on PC-3M cells.

2. Material and methods

2.1. Cell culture

PC-3M cell line, a human androgen-independent and highly metastatic CaP variant derived from PC-3 cell that was derived from bone metastases of patients with advanced CaP and reported to endogenously express PSCA mRNA [1], was purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China) and routinely maintained in RPMI 1640 medium (Hyclone, Logan, UT), supplemented with 10% fetal bovine serum (FBS, Hyclone), 100 units/ml penicillin G sodium, and 100 μg/ml streptomycin sulfate (Invitrogen, Carlsbad, CA). The cells were grown at 37°C in a humidified 95% air/5% CO_2 atmosphere. The culture medium was changed every other day and the cells were passaged when they reached 80% to 90% confluence.

2.2. Cell immunocytochemical staining

PC-3M cells were grown on poly-L-lysine-coated slides (Nalge Nunc, Rochester, NY). When the cells reached 80% confluence, the slides were washed in phosphate-buffered-saline (PBS) and then fixed with cold aceton for 15 minutes. Immunocytochemical staining for PSCA was conducted using a commercial kit (Boster Biological Technology Ltd., Wuhan, China), according to the manufacturer's instructions. Briefly, a primary rabbit anti-human PSCA antibody (Boster) with a 1:100 dilution was applied to incubate with the slides at room temperature for 2 hours. Labeling was detected by sequentially adding biotinylated secondary antibodies and streptavidin-peroxidase, and localized using 3,3′-diaminobenzidine reaction. Slides were then counterstained with hematoxylin. Substitution of the primary antibody with PBS served as a negative-staining control. The staining was visualized by CKX31-12PHP inverted microscopy (Olympus, Yokogawa, Japan).

2.3. Small interfering RNA transfection

The small interfering RNA (siRNA) targeting human PSCA (siRNA-PSCA) was purchased from Dharmacon Research Inc. (Lafayette, CO), and consisted of a set of two sequences (GCA AGA AGA ACA TCA CGT GTT and GTG ACA CCG ACT TGT GCA ATT). According to the published sequence of human PSCA gene (GenBank: NM_005672), these siRNA oligonucleotides were synthesized corresponding to the nucleotides 256–274 and 277–295 located 3′ to the first nucleotide of the start codon in the coding region of this gene. All designed siRNA sequences were submitted to a basic local alignment search tool BLAST search against the human genome database to ensure that only the PSCA gene was targeted. Scrambled siRNA oligonucleotide that does not target any gene was purchased from Dharmacon Research Inc. (Lafayette), and was used as the negative control siRNA.

PC-3M cells were seeded in a 24-well-plate at a density of 0.5×10^5 cells/well in 10% FBS containing RPMI 1640. The following day, when the cells reached 80%–90% confluence, they were transfected with 200 nmol/l siRNA for either the PSCA or the scrambled control using the Lipo-