Original Article

Prostate Specific Antigen Promoter-Driven Adenovirus-Mediated Expression of Both ODC and AdoMetDC Antisenses Inhibit Prostate Cancer Growth

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ABSTRACT

Objective: To generate recombinant adenovirus that could simultaneously express ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (AdoMetDC) antisenses specifically in prostate cancer cells, and evaluate its inhibitory effect on prostate cancer *in vivo*.

Methods: Fragments of ODC and AdoMetDC genes were generated by PCR, cloned into the pPGL-PSES, and then recombined with pAdEasy-1 vectors in AdEasy-1 cells. Ad-PSES-ODC-AdoMetDCas virus was produced in HEK293 cells. Following transfection with Ad-PSES-ODC-AdoMetDCas, the levels of ODC or AdoMetDC were determined by RT-PCR and western blot assays. The effect of Ad-PSES-ODC-AdoMetDCas treatment on tumor formation and growth was evaluated in xenograft models of prostate cancers *in vivo*.

Results: The plasmid pAdEasy-PSES-ODC-AdoMetDCas was successfully constructed and the recombinant Ad-PSES-ODC-AdoMetDCas adenovirus was produced. Transfection with Ad-PSES-ODC-AdoMetDCas adenovirus significantly inhibited the expression of ODC and AdoMetDC genes specifically in prostate DU145 cells, but not H1299, HT29 and HepG2 cancer cells, and disrupted the ability of DU145 cells to form solid prostate cancer *in vivo*. Intratumoral treatment with Ad-PSES-ODC-AdoMetDCas adenovirus significantly inhibited the growth of engrafted prostate tumors *in vivo*.

Conclusion: The recombinant Ad- PSES-ODC-AdoMetDCas adenovirus specifically reduces the expression of both ODC and AdoMetDC genes in prostate cells and may be used for treatment of prostate cancers at the clinic.

Key words: Prostate androgen independent promoter; Ornithine decarboxylase; S-adenosylmethionine decarboxylase; Adenovirus; Prostate cancer

INTRODUCTION

Prostate cancer is the most commonly diagnosed non-cutaneous neoplasm in men and the second leading cause of cancer mortality among American men[1]. Prostate specific antigen (PSA) is selectively expressed by benign, hyperplastic, and malignant prostate epithelium[2–4] and significantly increased levels of PSA in patient's sera is a valuable indicative of prostate diseases[5]. The incidence of prostate cancers is rapidly rising in developed countries due to increased sensitivity of PSA detection[4, 6]. The unique property of PSA expression allows to target therapeutic gene expression selectively in prostate tissues for the treatment of prostate cancers[7]. Indeed, previous studies have shown that the immediate 5' region of

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the PSA promoter was sufficient in driving the expression of interesting genes specifically in prostate tissue[8].

Polyamines, which belong to aliphatic compounds, exit in almost all living species and physiologically regulate the growth and differentiation of normal cells[9−11]. In mammalian cells, the intracellular polyamine biosynthesis is mainly regulated by two rate-limiting enzymes[12] ornithine decarboxylase (ODC) and S-adenosyl-methionine decarboxylase (AdoMetDC), which have been implicated in tumor growth by promoting the formation of more distal polyamines spermidine and spermine[13]. High levels of polyamine and elevated levels of polyamine synthetic enzyme activity have been detected in many kinds of cancers, including prostate cancer[14−17]. Indeed, inactivation of polyamine catabolism in Du145 prostate carcinoma cells attenuated their proliferation[18]. Therefore, down-regulation of ODC and AdoMetDC expression and depletion of polyamine content in prostate cancer by novel protocols of gene therapy may be a promising approach to treat prostate cancer[19].

In this study, we generated novel recombinant adenovirus using the PSA promoter controlled expression of two antisense RNAs for the ODC and AdoMetDC mRNAs, allowing disrupting the expression of ODC and AdoMetDC specifically in prostate epithelial cells. Its therapeutic efficacy in inhibiting the growth of prostate cancers was evaluated *in vivo*.

MATERIALS AND METHODS

Cells and Reagents

HT-29, H1299, HepG2 and Du145 cells were stored in our institute. HEK 293 packaging cells were purchased from Shanghai Institute of Biochemistry and Cell biology, Chinese Academy of Sciences (Shanghai, China).

The shuttle vectors of TA-ODC and TA-AdoMetDC were constructed in our laboratory. PGL3-PSES vector was a gift from Prof. Chinghai Kao in Indiana University (Indianapolis, IN). Escherichia Coli pAdTrack and pAdeasy-1 cells were stored in our laboratory.

Plasmids Constructions: Construction of pAdeasy-PSES-ODC-AdoMetDC

Plasmids TA-ODC and TA-AdoMetDC were used as the templates for the amplification of the ODC (140 bp) and AdoMetDC (220 bp) gene fragments by polymerase chain reaction (PCR). The target fragments were purified after agarose-electrophoresis and inserted into the pMD19-T simple vector, achieving the plasmid, designated as TA-ODC-AdoMetDC. Subsequently, the designed 360 bp fragment was further cloned into the Kpn I and EcoR V sites of the pAdTrack null vector, which generated the recombinant plasmid pAdTrack-ODC-AdoMetDC. Next, the Hind III DNA fragment of pAdTrack-ODC-AdoMetDC was further inserted into the corresponding site of the pPGL-PSES (bearing prostate androgen independent promoter) vector and formed pAdTrack-PSES-ODC-AdoMetDC. Finally, pAd-Track-PSES-ODC-AdoMetDC was linearized by digestion with Pem I, purified, and then transformed (1 μl linearized plasmid DNA) into highly competent cells, AdEasy-1, for generating recombinant plasmid pAdeasy-PSES-ODC-AdoMetDC. Its authenticity was confirmed by DNA sequencing.

Viral Preparation and Analysis

The recombinant plasmid pAdeasy-PSES-ODC-AdoMetDC was linearized with Pac I, and transformed into 293 packing cells for the generation of recombinant adenovirus, Ad-PESE-ODC-AdoMetDCas, using Lipofectamine 2000 (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions. Sixteen hours post transfection, the cells were harvested and lyzed by freezing and thawing repeatedly, followed by centrifuging at 10,000 rpm for 10 min for collecting the viral supernatant. The recombinant viral particles were purified by ultracentrifugation in cesium chloride step gradients. The genes contained in the virus were analyzed by RT-PCR. The titers of purified adenoviruses were measured by green fluorescent protein (GFP) expression.

Evaluation of Gene Transduction Efficiency *In Vitro*

HT-29, H1299, HepG2 or Du145 cells at 7×10^4/well were infected with Ad-PESE-ODC-AdoMetDCas at multiplicities of infection (MOI) of 30, 50, 70, 90, or 110, respectively, for 24 h. The GFP-positive cells were quantified under a fluorescence microscope.

PT-PCR Analysis of ODC and AdoMetDC mRNA Levels

Following infection with the Ad-PESE-ODC-