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Highly specific expression of luciferase gene in lungs of naïve nude mice directed by prostate-specific antigen promoter

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Abstract

PSA promoter has been demonstrated the utility for tissue-specific toxic gene therapy in prostate cancer models. Characterization of foreign gene overexpression in normal animals elicited by PSA promoter should help evaluate therapy safety. Here we constructed an adenovirus vector (AdPSA-Luc), containing firefly luciferase gene under the control of the 5837 bp long prostate-specific antigen promoter. A charge coupled device video camera was used to non-invasively image expression of firefly luciferase in nude mice on days 3, 7, 11 after injection of 2×10^9 PFU of AdPSA-Luc virus via tail vein. The result showed highly specific expression of the luciferase gene in lungs of mice from day 7. The finding indicates the potential limitations of the suicide gene therapy of prostate cancer based on selectivity of PSA promoter. By contrary, it has encouraging implications for further development of vectors via PSA promoter to enable gene therapy for pulmonary diseases.

Keywords: Prostate-specific antigen promoter; Non-invasive imaging; Specific expression; Lungs; Naïve nude mice; Prostate cancer; Gene therapy; Adenovirus; Luciferase; Pulmonary diseases

Prostate cancer is the most common malignancy diagnosed and the second leading cause of cancer death in men in the United States [1]. Principal treatments in patients with advanced prostate cancer are based on androgen ablation strategies but most such patients tends to have hormone refractory disease later in the course of treatment [2]. Recent advances in molecular biology offer new hope in the form of gene therapy. A popular cytotoxic gene therapy approach is gene-directed enzyme prodrug gene therapy, also known as suicide gene therapy [3,4]. Its aim is to avoid systemic toxicity, due to the lack of tumor specificity of existing cytotoxic agents, by ensuring that they are generated in high concentrations only at the tumor site, thereby, increasing the therapeutic index [5]. Selectivity and efficacy are the most critical factors in such successful suicide gene therapy.

In that context, tumor- or tissue-specific promoters have been used to target tumor cells selectively. Prostate-specific antigen (PSA) is expressed primarily in both normal prostate epithelium and the vast majority of prostate cancers. Increase in serum PSA during endocrine therapy are generally considered as evidence for prostate cancer recurrence or progression to androgen independence [6]. Target-specific expression of therapeutic toxic genes in tumor cells through the use of tissue-specific promoters could decrease their toxic effect on neighboring normal cells, when virus-mediated gene delivery results in their infection. The PSA promoter has been demonstrated the utility for tissue-specific toxic gene therapy for prostate cancer [7]. But the expression of naïve PSA and the foreign gene overexpression elicited by PSA promoter has not well determined in normal animals although it is an important issue for therapy safety.

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The cooled CCD optical imaging belongs to a new generation of in vivo imaging technologies that use fluorescent or bioluminescent reporter genes to produce a signal from within a living animal. The CCD approach detects low levels of luminescence consistently and reproducibly from fur-covered animals without the need for an external light source [8,9]. CCD camera has been used to non-invasively image luciferase-expressing human prostate tumors and metastases in nude mice [10]. Here we employed the CCD imaging system to image luciferase expression in normal nude mice after tail-vein injection of a prostate-specific adenovirus vector (Ad-PSA-Luc).

Materials and methods

Cell lines and cell cultures. C4-2 was established from LNCaP tumors propagated in castrated hosts [11,12], and WH was established from a human bladder transitional cell carcinoma [13]. All cell lines were maintained in T-medium (Invitrogen, Carlsbad, CA), with 5% fetal bovine serum (FBS; Invitrogen).

Construction of recombinant adenoviruses. All plasmid constructs were prepared using standard methods [14]. The original pPSA-Luc containing the 5.8-kb PSA promoter was obtained from Dr. Leland W.K. Chung. It was generated by inserting a *Hin*dIII fragment of the PSA promoter in the multiple cloning site (MCS) of pGL3-basic vector (Promega, Madison, WI). pShuttle-PSA-Luc was generated by inserting a *Kpn*I/*Sal*I fragment of pPSA-Luc, which contains PSA promoter, luciferase gene, and SV40 late poly(A) signal, in the MCS of pShuttle vector [15].

AdEasy Vector System [15] was used for construction of recombinant adenovirus of the firefly luciferase under the control of the 5837 bp prostate-specific antigen promoter. Briefly, pShuttle-PSA-Luc was linearized with *Pme*I and co-transformed into *Escherichia coli* strain BJ5183 together with pAdeasy-1, the viral DNA plasmid. The pAdeasy-1 is E1 and E3 deleted, its E1 function can be complemented in 293A cells. The recombinant adenoviral construct, which was named pAd-PSA-Luc was then cleaved with PacI to expose its inverted terminal repeats (ITR) and transfected into 293A cells to produce viral particles. The recombinant virus was identified with restriction analysis, PCR, RT-PCR, and detection of luciferase activity. The recombinant virus Ad-PSA-Luc was purified through two cesium chloride gradients, and then purified virus was desalted by dialysis at 4 °C against 10 mM Tris–hydrochloric acid buffer with 10% glycerol and stored in aliquots in liquid nitrogen. The titer of virus preparations was determined by plaque assay according to the application manual of the pAdEasy Vector System.

As a control, AdCMV-Luc, which contains CMV promoter and luciferase gene, was constructed as described above.

Evaluation of expression of AdPSA-Luc in prostate and non-prostate cancer cells. Cells C4-2 or WH at ~80% confluency in 24-well plates were infected with AdPSA-Luc or AdCMV-Luc at the specified PFU per cell. From 24 h after transduction, an aqueous solution of substrate, D-firefly lucifern (Xenogen, Alameda, CA) was added into cells (5 μl/well, 15 mg/ml) and the imaging was performed by an IVIS CCD camera (Xenogen) [8].

In vivo gene transfer. Six- to eight-week-old male athymic nude mice (nu/nu strain [Balb/c background], Harlan Sprague–Dawley, Indianapolis, IN) were used for all in vivo experiments. They were kept under pathogen-free conditions in laminar flow boxes in accordance with established institutional guidelines and approved protocols. Adenoviruses (2.0×10^9 PFU) AdPSA-Luc and AdCMV-Luc were injected into naïve nude mice ($n = 4$) , respectively, via tail vein.

CCD imaging to detect in vivo luciferase expression. On days 3, 7, and 11, the CCD images were obtained using a cooled IVIS CCD camera (Xenogen) and images were analyzed [9]. Light was monitored in all of the experiments described at 5 min after injection of luciferin. The CCD signals were quantified as total relative light units per minute of acquisition time (RLU/min) in the region of interest (ROI). On day 11, mice were sacrificed and isolated organs were imaged.

Results and discussion

Tissue-specific expression of AdPSA-Luc in human cancer cell line

We constructed an adenovirus vector (AdPSA-Luc), which can express firefly luciferase under the control of the 5837 bp prostate-specific antigen promoter (Fig. 1).

The tissue-specific expression of AdPSA-Luc was tested by CCD camera imaging in human prostate cancer PSA-producing cell line C4-2, an androgen-independent subline of LNCaP, and non-prostate cancer cell line WH. Fig. 2A shows that AdPSA-Luc led to significantly higher level of luciferase expression in C4-2 than in WH on days 2 after transduction when they were transduced at 80, 40, 20, 10, 5, and 2.5 PFU of AdPSA-Luc per cell. We imaged the infected cells from 2 to 5 days after transduction and the similar results were obtained. The infectivities of C4-2 and WH were compared by using the same range of AdCMV-Luc as the infecting viruses (Fig. 2B).

The high tissue-specificity of the long PSA promoter has been well determined in its recombinant plasmids, transgenic animals as well as adenoviral vectors [7,16,17]. Our results are consistent with these publications. In contrast, Shi et al. [18] reported that the PSA promoter cassette in helper-dependent adenoviral

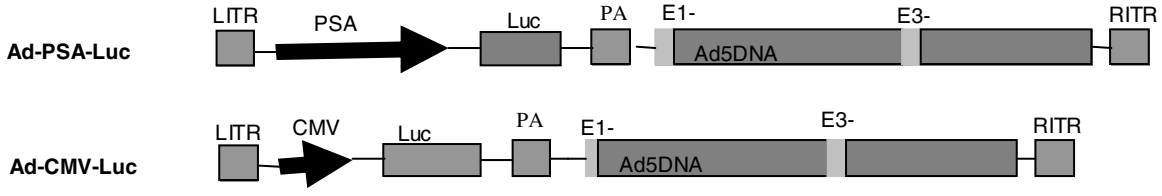

Fig. 1. Schematic representation of Ad vectors used in this study. ITR, Ad inverted terminal repeats; CMV, human cytomegalovirus promoter; PSA, prostate specific antigen promoter; Luc, firefly luciferase gene.