Ad5-(PSE-BC)-(GAL4-$(VP16)_2$)-$(GAL4)_5$-Fluc

AdTSTA-FL

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Chemical name:	*Ad5*-(PSE-BC)-(GAL4-$(VP16)_2$)-$(GAL4)_5$-Fluc	
Abbreviated name:	AdTSTA-FL	
Synonym:		
Agent category:	Protein (Virus)	
Target:	Androgen receptor	
Target category:	Receptor	
Method of detection:	Optical imaging	
Source of signal/contrast:	Luciferin	
Activation:	Yes	
Studies:	• *In vitro* • Rodents	No structure is currently available in PubChem.

Background

[PubMed]

Adenoviruses (Ads) comprise a non-enveloped icosahedral protein shell (capsid) 70–100 nm in diameter surrounding an inner vial genome core (1). Human Ads consist of 51 distinct serotypes that are classified into six subgroups (species) designated A–F (2). Some serotypes such as serotypes 2 (Ad2) and 5 (Ad5) of species C only induce a mild, non-oncogenic respiratory infection, making them suitable for development of Ad-based vaccines and gene delivery vehicles for systematic administration (1). Their core genome, a linear, double-stranded DNA of ~36 kb, contains five early transcription units (E1A, E1B, E2, E3, and E4), four intermediate units (IVA2, IX, VAI, and VAII), and one later transcription unit (2). The functions in the early transcription regions have been well identified,: E1A activates cell cycles and initiates DNA replication; E1B blocks apoptosis

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induced by E1A activity; E2 facilitates viral DNA replication; E3 modulates host immune responses; and E4 regulates DNA replication, mRNA transport, and apoptosis (3). As a gene delivery vehicle, the genome core in Ad2 or Ad5 is usually modified with recombinant transcription activators (RTAs), in which foreign DNA with a size of up to 7.5 kb can be inserted in the deleted E1 or E3 regions in the Ads (4). Such genetic modification can incorporate ligands that can recognize specific cellular receptors and/or block the adenoviral naïve receptor (coxsackie-adenovirus receptor (CAR)), so that their tissue specificity is enhanced significantly. For example, the gene for the androgen receptor (AR) regulates the growth of prostate epithelial cells, induces the expression of prostate-specific antigen (PSA), and controls the growth of prostate cancer in the early phase. The AR gene can be introduced to the adenoviral genome to target AR-responsive prostate cancer or metastasis (5). Furthermore, reporter genes such as firefly luciferase (Fluc) (6) can be included for *in vivo* imaging, or suicide genes such as herpes simplex virus type 1-thymidine kinase (HSV1-tk) can be used for therapeutic applications (7). As an oxygenase (Mw = 62 kDa), Fluc oxidizes the heterocyclic substrate D-luciferin to oxyluciferin injected into the body and emits light in the wavelength range of 400–620 nm (8).

The transcriptional activity of adenoviral genes can be amplified several orders of magnitude with the use of the GAL4-VP2 fusion protein, where GAL4 is a DNA-binding domain (147 amino acids) and VP2 is a two-tandem repeat of the herpes simplex virus VP16 acidic activation domain (78 amino acids) (9). The two-step transcriptional amplification (TSTA) system is a novel recombinant transcription activation approach for designing a PSA promoter/enhancer, which can provide as high as ~800-fold enhancement in transcription activity compared with its conventional analog (10, 11). The TSTA system is composed of two parts in general; i.e., an activator BC-VP2 that contains a PSA promoter with a duplicated ARE4 enhancer core (each core has four binding sites for AR) to control the expression of the chimeric protein GAL4-VP2 and provide cell-specific binding, and the reporter $(GAL4)_5$-Fluc (G5-FL), which contains a reporter with five GAL4 repeats to generate a GAL4-responsive Fluc signal (6). The two parts are linked in a head-to-head orientation and are inserted into the E1 region of Ad5 to produce *Ad5*-(PSE-BC)-(GAL4-$(VP16)_2$)-$(GAL4)_5$-Fluc (AdTSTA-FL), an optical agent for imaging AR-responsive PSA. Such a construct can provide ~50-fold amplification (11) *via* two steps: the PSA regulates the expression of the potent transcription activator GAL4-VP2, which in turn activates a GAL4-responsive reporter Fluc. AdTSTA-FL can function effectively as a gene delivery vehicle and as a lymphotropic agent in prostate cancer and metastasis *in vivo* (6, 12).

Synthesis

[PubMed]

The preparation of AdTSTA-FL was conducted in several steps (5, 6, 10, 11, 13). The commercial plasmid pBS II SK+ was used as the starting baseline construct pPSE (11). A *Not*I-flanked PSA enhancer/promoter expression cassette derived from plasmid

PSAR2.4k-PCPSA-P-Lux was inserted into the pPSE to produce PSE. The synthetic ARE4 element that contained four key ARE elements was used to generate a duplicated enhancer core (13). The insertion of this duplicated core (C) in the PSE replaced the sequence between -3935 and -2855 in the PSE to produce a plasmid vector PBC. Then a GAL-VP2 fragment was inserted into PBC followed by creation of a unique *Not*I site. A *Not*I fragment, which contained both the PBC promoter and GAL-VP2 gene, was excised from the obtained plasmid PBC-VP2 and cloned into the *Not*I site of G5-FL to generate the vector PBC-VP2G5-FL (10). A *Sal*I-*Not*l fragment containing the PBC-VP2G5-FL fragment was derived from the resulting PBC-VP2G5-FL and excised by *Not*I and partial *Sal*I digestion (5). The produced expression cassette PBC-VP2G5-FL was inserted into the *Sal*I-*Not*I site of the commercial vector pShuttle, which was then incorporated into the commercial Ad vector AdEasy through homologous recombination to produce AdTSTA-FL (6). The virus was propagated in 293 cells, purified on a CsCl gradient, and titered with plaque assays on 293 monolayers.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Zhang et al. examined the activity of AdTSTA-FL *in vitro* (5). Androgen-dependent prostate cancer cells (LNCaP) were infected with AdTSTA-FL and incubated up to 48 h. The cells were harvested and lysed for assaying activity. GAL4-VP2 and Fluc levels were enhanced significantly in the presence of the synthetic androgen agonist R1881, with a maximum enhancement at 48 h after transcription. Similar studies were conducted in other cells, including MCF7 cells (androgen-expressing breast cancer cells) and PC3 (androgen-negative prostate cancer cells), but only a low basal level of TSTA expression was observed and did not respond to R1881. Thus the AdTSTA-FL responded only to androgen-dependent prostate cancer cells. AdTSTA-FL displayed 10-fold more ligand-induced Fluc activity than the control, AdCMV-FL.

Sato et al. examined the specificity of AdTSTA-FL *in vitro* on the basis of the measurement of infectivity (6). A variety of cells were used in the study, including LNCaP and LPAC-4 cells, H157 and A549 cells (lung cancer), MCF7 cells, and HepG2 cells (liver cancer). The infectivity in these cells with respect to that in the Hela cells (cervical carcinoma) was evaluated on the basis of their viral DNA uptake, which was 1.7 for LNCaP, 1.6 for H157, 1.5 for MCF7, and 1.1 for LAPC-4 and HepG2. Then the activity of AdTSTA-FL was evaluated in several prostate cancer cell lines, including two androgen-responsive cells (LNCaP and LPAC-4) and two AR-negative cells (DU145 and PC3). With a dose of one plaque-forming unit (pfu, an infectious unit) per cell (1 multiplicity of infection (m.o.i)), the normalized Fluc activity was 4.4-fold lower in LPAC-4 than that in LNCaP, whereas the AR-negative cells exhibited negative results (i.e., ~500-fold lower activity). The luciferase activity in other cancer cells was also measured and normalized to the activity measured for LNCaP, and all had much lower activity: a reduction of 2.9-fold was found in A549, 12-fold in H157, 45-fold in HepG2, 60-fold in Hela, and 200-fold in MCF7.

Burton et al. assessed the accumulation of AdTSTA-FL in lymph nodes *ex vivo* (12). Mice ($n = 3$) were injected with 1×10^8 pfu AdTSTA-FL in 20 µl phosphate-buffered saline at their forepaws. Their draining lymph nodes (the axillary and brachial nodes) were dissected 24 h after injection. The nodal capsules were disrupted for extraction of the adenoviral particles. The transduction luciferase activity was found in ipsilateral axillary and brachial lymph nodes but not in the contralateral nodes. The number of viral genomes transported to and retained in the brachial node was ~60,000 copies, which was equivalent to ~0.1% delivery efficiency. Burton et al. also examined the transverse of AdTSTA-FL to regional lymph nodes in the mouse prostate *Pten* tumor suppressor gene knockout model ($Pten^{-/-}$). Ten-week-old $Pten^{-/-}$ mice with enlarged carcinoma prostate glands were injected with 1×10^8 pfu AdTSTA-FL on their dorsolateral lobe. One hour later, ~1% of injected AdTSTA-FL was found in the periaortic lumbar nodes, with a smaller amount in more distant mesenteric and renal lymph nodes.

Animal Studies

Rodents

[PubMed]

Sato et al. investigated the specificity of AdTSTA-FL *in vivo* (6). SCID mice ($n = 4$, ~25 g) were implanted with LAPC-4 xenografts, and the tumors were allowed to grow for 3 weeks. AdTSTA-FL at a dose of 10^7 pfu in 10 µl was injected into each tumor. A cooled-charged-coupled device (CCD) camera was used to image the *in vivo* expression over a 22-d period. For each imaging session, the mice were intravenously injected with 150 mg/kg D-luciferin (200 µl). A robust signal was observed 4 d after injection of AdTSTA-FL. Compared with the injection of the control AdCMV-FL, the average activity was 110-fold higher. As a control, naïve mice were used for the study. A low signal appeared only in the lung 22 d after intravenous injection of AdTSTA-FL. The kinetics of the expression after intratumoral injection of AdTSTA-FL was examined with sequential images between day 5 and day 13. The AdTSTA-FL displayed 50- to 100-fold higher levels of Fluc activity than the AdCMV-FL, and both gradually decayed from day 7.

Burton et al. examined the movement of AdTSTA-FL to lymph nodes *in vivo* (12). SCID/Beige (natural killer–deficient) mice were implanted with LAPC-9-VEGF-C tumor cells in the right shoulder, and the tumors were allowed to grow to 1.5 cm in diameter. Tumor-bearing mice and naïve mice were injected with 1×10^8 pfu AdTSTA-FL in 20 µl phosphate-buffered saline in their forepaw and imaged with a CCD camera 4 d later. In each imaging session, the mice received 150 mg/kg D-luciferin intraperitoneally 20 min before imaging. A clear Fluc signal was observed in the axillary region of the tumor-bearing mice but not in the naïve mice. Burton et al. further assessed the lymph node metastases in nodal lesions of various sizes (12). Mice ($n = 6$) bearing 1-cm LAPC-9-VEGF-C-GFP-RL tumors were used for the study of macroscopic nodal lesions. A strong signal was observed in the ipsilateral axilla of mice with an injection of 1×10^8 pfu AdTSTA-FL in both paws. Mice with orthotopic implanted LAPC-9-VEGF-C-GFP-RL

tumors were used to examine the extensive peritumoral lymphatics. Fifteen days after tumor implantation, the mice were injected with 1×10^8 pfu AdTSTA-FL in both hind paws. An observable luciferase signal was found in the inguinal and periaortic region 4 d later. The regional lymph nodes were excised for histological analysis after imaging, which further confirmed the metastasis lesion (~250 μm) of tumors present in the lymph nodes. As a control, luciferase tagged Ads that used cytomegalovirus (CMV) as promoter (AdCMV-FL) were injected in the forepaw of tumor-bearing mouse. The bioluminescence images demonstrated high nonspecific signal at the site of injection (paw) and in the liver caused by the systemic circulation.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

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