

## Review

# Tropism and transduction of oncolytic adenovirus vectors in prostate cancer therapy

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## 1. Abstract

Oncolytic adenovirus has been applied in cancer therapy because of several advantages such as cost-effective production, high transduction efficiency and low toxicity. Recent efforts have been focused on the modification of oncolytic adenovirus by encoding transgenes within the viral genome to efficiently and selectively replicate within cancer cells, destroy cancerous cells, induce tumor cell apoptosis, and stimulate the recruitment of immune cells to the tumor site. Nevertheless, there are still big challenges for translational research of oncolytic virotherapy in clinical cancer management. Therefore, here we summarize current status on the design and application of oncolytic adenovirus vectors for prostate cancer therapy. In particular, we describe the main receptors associated with the tropism and transduction of oncolytic adenovirus vectors, and propose new directions in future studies for prostate cancer virotherapy.

## 2. Introduction

Prostate cancer (PCa) is a common cancer in the male worldwide, and most of PCa can be cured by surgery or radiotherapy in the early stage [1]. However, up to 15% of initial patients are diagnosed to develop metastatic lesions, and recurrence rate of patients after conventional radical therapy is more than 40% [2]. Androgen deprivation therapy (ADT) has been developed for recurrent PCa, but some patients still relapse because of the progression of castration-resistant prostate cancer (CRPC) [3]. Since traditional therapies including chemotherapy and radiotherapy are not highly effective and have obvious side effects of cytotoxicity for CRPC, novel therapeutic strategies are urgently needed. Oncolytic adenovirus as a new viral therapy agent for CRPC has gained increasing attention due to several advantages such as high selectivity, low cytotoxicity and oncolysis characteristics.

Adenovirus (Ad) has been the workhorse of vi-

rotherapy since 1950s. Unfortunately, due to the rapid development of chemotherapy and safety concern of virotherapy, the enthusiasm for virotherapy suddenly faded away later [4]. Only recently, virotherapy has become a hot topic with the development of Ad and retroviral vectors for the delivery of a variety of transgenes targeting different types of cancers [4]. Therefore, in this review we summarize current status on the design and application of oncolytic Ad vectors for PCa therapy.

### 3. Genome structure of Ad

To develop Ad vector as a novel approach of cancer therapy, it is important to understand the genome structure of Ad in order to design recombinant Ad vectors. Ad is a non-enveloped virus with double-stranded DNA genome. Total 103 Ads can be divided into seven 'species' named A to G based on their genotypes [5]. Among them, A and C species are the most prevalent, and Ad serotype 5 (Ad5) is mainly used in the studies on Ad [6].

There are five early transcription units (*E1A*, *E1B*, *E2*, *E3* and *E4*), two delayed transcription units (*IX* and *Iva2*) and one late transcription unit (*L1-L5*) in Ad 5 coding region [7]. *E1A* conserved region 2 (*E1A*-CR2) interacts with retinoblastoma (Rb) in host cells to promote S-phase entry and viral DNA replication [7]. *E1B* contains *E1B-19K* and *E1B-55K*. *E1B-19K* is a functional *Bcl-2* homologue and plays a dual role in apoptosis and autophagy [8]. *E1B-55K* could promote virus survival in tumor cells, but is not necessary for oncolytic effects of adenoviruses [9]. *E2* region encodes polymerase, DNA binding protein (DBP) and preterminal protein (pTP), which are important for viral transformation and replication. *E3* region encodes adenovirus death protein (ADP), which promotes the cytolysis of host cells and the spread of the virus to the surrounding cells [10]. *E4* region plays an important role in transition and late viral gene expression, and is vital for viral replication and virion assembly (Fig. 1).

Ad5 is categorized into two vectors based on oncolytic character: conditionally replicating adenoviral (CRAd) vector which only propagates and lyse cancer cells, and could not replicate in normal cells; and replication-defective adenoviral (RDAd) vector with the deletion of *E1*, which could not replicate in cells but can carry therapeutic genes [11].

### 4. Main receptors for oncolytic adenovirus

To achieve optimal anti-tumor efficacy of Ad vector, we need develop Ad vector with improved tropism and transduction. Therefore, we need identify cellular receptors that mediate the tropism and transduction of Ad. Cell attachment of oncolytic adenovirus is initiated by the attachment of the fiber protein to CAR receptor [12]. Next, the interaction between Arg-Gly-Asp (RGD) motifs in Ad

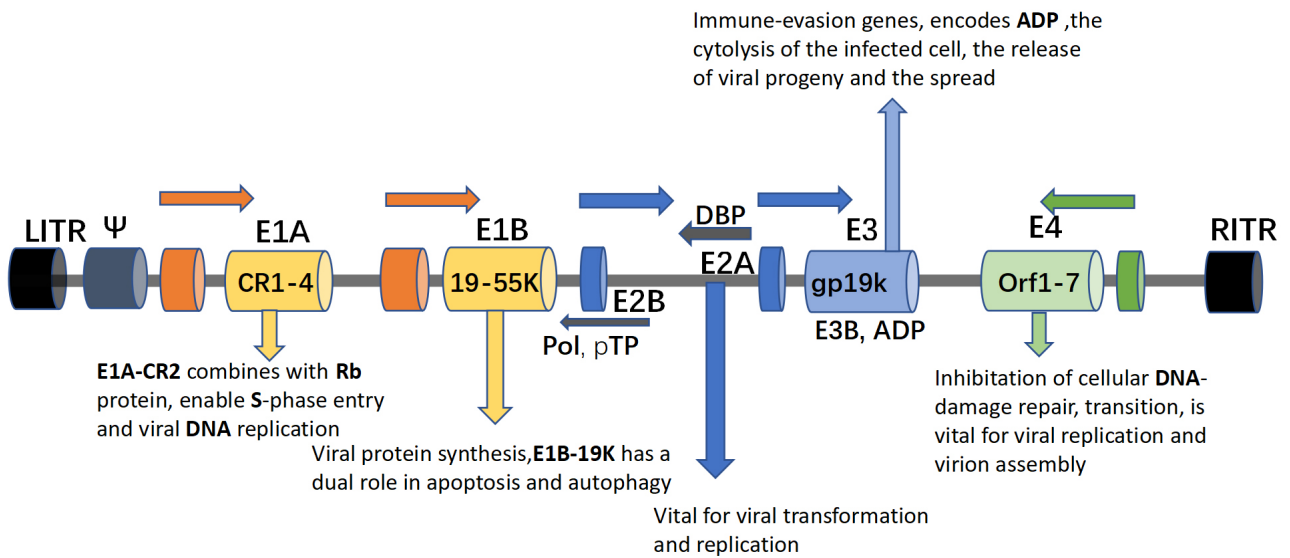
penton-base protein and  $\alpha v\beta 3$  and  $\alpha v\beta 5$  integrin triggers virus internalization. Intravenous administration of Ad vectors is hindered by viral neutralizing antibodies (nAbs), other proteins in the circulating blood, inefficient transduction and hepatotoxicity [13]. It is reported that nAbs mainly hinder systemic administration of oncolytic Ads in preclinical and clinical studies [14]. In addition, Ad5 efficiently binds to lymphocytes and erythrocytes [15, 16]. Therefore, nonspecific tumor-selectivity and vector transduction are the main challenges, which may cause hepatotoxicity and low efficiency in specific tumor delivery.

Both CAR and  $\alpha v\beta$  integrin are the tropism determinants of Ad5 [17]. The liver is susceptible to the hepatotoxicity of Ad5, which may be due to high expression of CAR in the liver although CAR expression is low in PCa [18]. A novel Ad5 vector containing two amino acid mutations in the AB loop of the fiber-modified Ad5 fiber-knob reduced liver tropism and increased the anti-tumor efficacy of the vector in low CAR expression or CAR deficient cancer cells following intravascular delivery [19]. Therefore, CAR-independent targeting strategy has promise for the treatment of CAR deficient PCa.

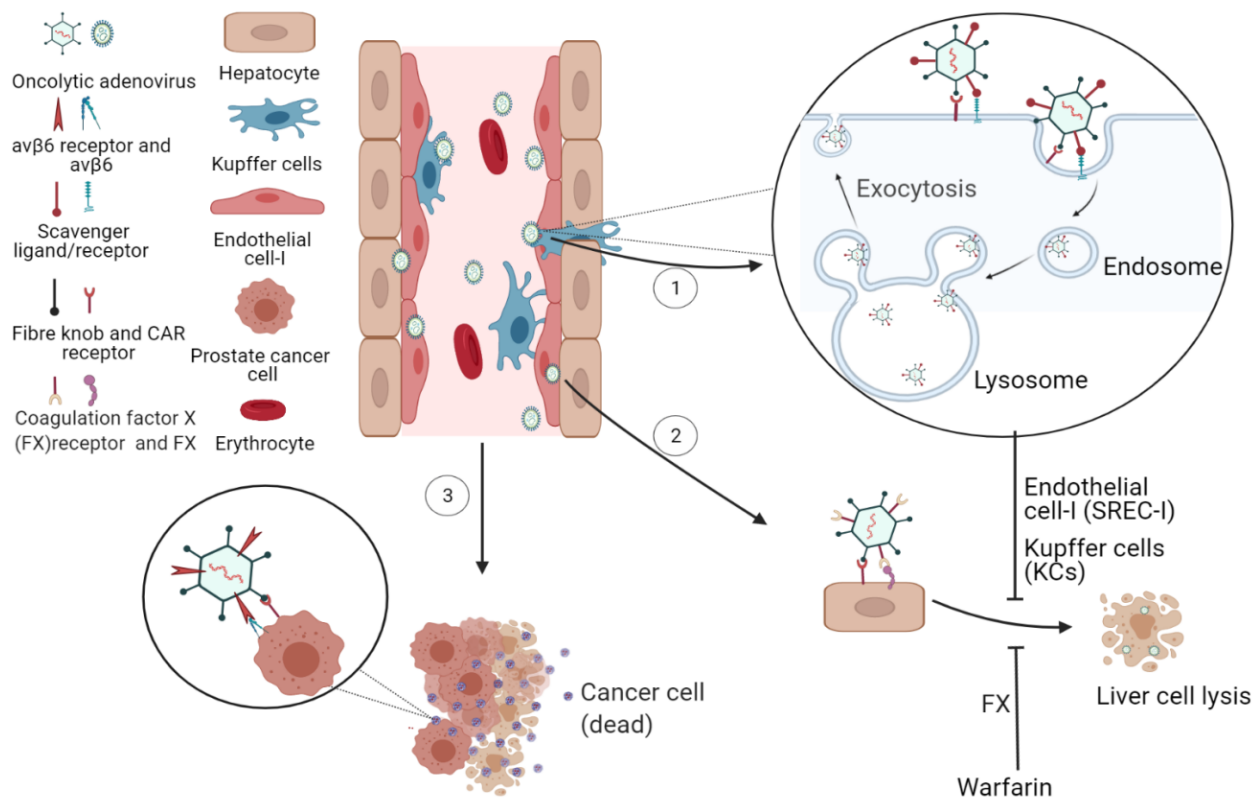
The upregulation of  $\alpha v\beta 6$  integrin has been suggested to correlate with tumor progression [20, 21]. A trial targeting  $\alpha v\beta 6$  integrin was conducted. Ad5.HI.A20 and Ad5/kn48.DG.A20 were generated by inserting A20 (a 20-amino acid peptide) into penton base protein loop targeting  $\alpha v\beta 6$  integrin, leading to 160 and 180 fold increase in the transduction of BT-20 breast carcinoma cells with high expression of  $\alpha v\beta 6$  [22].

Coagulation factor X (FX) is an adapter for coagulation factors involved in liver tropism following systemic delivery [23]. Ad5 recognizes FX via hexon hypervariable region, and FX then interacts with heparan sulfate proteoglycans (HSPGs) on the surface of liver cells [24]. Ablating the binding of FX to Ad5 can diminish virus localization to the liver. Warfarin pretreatment significantly reduced liver sequestration and hepatic toxicity of Ad vectors [25].

On the other hand, Ad shows high affinity to scavenger receptor-A (SR-A) and scavenger receptor expressed on endothelial cell-I (SREC-I). Kupffer cells (KCs) and liver sinusoidal endothelial cells (LSECs) recognize Ad and remove them from the circulation to inhibit efficient hepatocyte transduction (Fig. 2). The efficiency of hepatocyte transduction by Ad could be increased by blocking SR-A and SREC-I [26]. Two peptides PP1 and PP2 have been designed to block SR-A and SREC-I, respectively, and they significantly improved Ad-mediated hepatocyte transduction efficiency [27].



**Fig. 1. Illustration of the structure and function of Ad5 genome.** LITR and RITR indicate left and right inverted terminal repeats, respectively. Abbreviation: E1A-CR2, E1A conserved region 2; Rb, retinoblastoma; ADP, adenovirus death protein; DBP, DNA-binding protein; pTP, precursor terminal protein; Pol, polymerase.



**Fig. 2. The ligands and receptors involved in Ad tropism and transduction.** (1) Ad is picked by SR-A and SREC-I on KC and LSEC, and then is internalized through pinocytosis and then passed to lysosome. Most Ads are released from hepatocyte by first-pass effect which reduces the tropism and transduction and protects the hepatocytes from lysis. (2) Ad binds to FX and then FX binds to HSPGs on liver cells. Warfarin significantly reduces liver sequestration and hepatic toxicity. (3) Ad binds to CAR on PCa cells and then is internalized via  $\alpha v \beta 6$ . Ad replicates in PCa cells and induces cells lysis. Abbreviation: Ad, adenovirus; SR-A, scavenger receptor-A; SREC-I, scavenger receptor expressed on endothelial cell-I; KCs, Kupffer cells; LSECs, liver sinusoidal endothelial cells; FX, Coagulation factor X; HSPGs, heparan sulfate proteoglycans; CAR, coxsackievirus and adenovirus receptor; PCa, prostate cancer.

## 5. Development of oncolytic adenoviruses for prostate cancer therapy

### 5.1 Incorporating or deleting special genes

To improve the potency of oncolytic adenovirus, incorporating exogenous genes or deleting genes of adenovirus backbone is the first approach. For example, *p53* gene, a well-known pro-apoptosis gene, was introduced into Ad to generate Ad-p53 to induce cell death pathways in tumor tissues [28]. Oncolytic mutant Ad $\Delta\Delta$  vector with the deletion of *E1B19K* and *E1ACR2* exhibited potent effects to induce apoptosis of prostate cancer cells [29].

### 5.2 Prostate-specific promoter

The incorporation of prostatespecific promoter/enhancer leads to virus replication and the induction of the expression of exogenous genes only in prostate cancer cells [8]. Prostate specific antigen (PSA) promoter has been utilized in Ad mediated gene therapy against PSA-positive PCa. For example, Ad/PSAP-GV16- $\beta$ G vector was developed for combined use with prodrug DOX-GA3 to kill LNCaP cell xenograft tumor in nude mouse model [30].

Prostate specific membrane antigen (PSMA) is primarily expressed in PCa cells and highly expressed during PCa metastasis [31–33]. PSMA promoter based Ad vector Ad-PSMA (E-P)-CD drove the expression of cytosine deaminase and efficiently kill PSMA-producing CL-1 xenograft tumor with combined use of prodrug 5fluorocytosine [34].

PB promoter as a prostate-specific promoter was also utilized. Ad-ARR2PB-Bax vector contained PB promoter and two androgen response elements (ARR). PB promoter drove the expression of pro-apoptotic *Bax* and induced apoptosis in LNCaP xenograft tumor. Therefore, PB promoter based Ad vector has potential to target AR positive PCa [35].

Prostate cancer gene 3 (PCA3/DD3) is a PCaspecific marker identified recently. Hao *et al.* [36] designed OncoAd.mK5.DD3 vector to drive the expression of *mK5* (the mutational kringle5 of human plasminogen) by DD3 promoter specially in PCa cells and the results showed that OncoAd.mK5.DD3 was able to inhibit PCa efficiently. Taking advantages of the sensitivity and specificity of DD3 as PCa marker, a test kit Progenesa™ (Gen-Probe Inc. San Diego, CA, USA) was developed for the detection of PCa cells in urine [37].

### 5.3 Enhance the tropism and transduction of Ad

Ad5 recognizes coxsackievirus and adenovirus receptor (CAR) on host cells via fiber protein. Therefore, the modification of fiber could change virus tropism [38]. The incorporation of RGD motif in fiber protein into AdRGD-PGp53 led to enhanced transduction in PCa cells and upregulation of *p53* expression, with effective anti-tumor activity

both *in vitro* and *in vivo* [39]. On the other hand, Ad5/3-CTV oncolytic virus was engineered with the change of Ad.5 fiber knob to Ad.3 fiber knob, and it facilitated virus infection in a CAR independent manner, showing higher efficiency in human PC cells with low CAR expression [40].

### 5.4 Enhance immunotherapy

Since anti-tumor effect of Ad is partially mediated by virus induced immune response, oncolytic immunotherapy gains more attention recently. An oncolytic Ad (Ad5-yCD/mutTKSR39rep-mIL12) was designed to express pro-inflammatory cytokine IL-12 and suicide gene, the high anti-tumor efficacy provided the support for further development of this approach in clinical trials [41].

GM-CSF (Granulocyte-macrophage Colony Stimulating Factor) is an immune-modulatory cytokine that induces the activation of monocytes and macrophages, and promotes T-cell mediated anti-tumor response [42]. Ad5 $\Delta$ 24/3-RGD-GM-CSF with the expression of *GM-CSF* exhibited potent anti-tumor effects in PCa, and it induced tumor cell death and activated T-cells in response to antigen presentation by the exposure of tumor antigen [43]. A replication-selective Ad vector Ad5/3- $\Delta$ 24-GM-CSF was designed and applied in 21 patients with advanced solid tumors refractory to standard therapies. All patients had no severe adverse events. Virus activity was observed in 13/21 patients and 8/12 patients showed clinical benefit based on the evaluation with Response Evaluation Criteria In Solid Tumors (RECIST) criteria [44].

The combination of Ad with CD40L-based costimulatory molecule induced both humoral and cellular immunity against many types of cancer. Ad-PL-PPT-E1A was constructed with the fusion of *PSA* and *CD40L* and it exhibited enhanced anti-tumor activity, which could be a promising approach for gene therapy of advanced PCa [45].

Combination therapy with immune checkpoint blockade efficiently kills tumor [46]. PD-L1 inhibits T cell function against solid tumors, which may decrease anti-tumor effect of chimeric antigen receptor-modified T cells (CAR T-cells) [47]. Therefore, Ad vector engineered to express PD-L1 blocking antibody has become a strategy to enhance anti-tumor efficacy of CAR T-cells [48].

### 5.5 Autologous cells as carriers

To avoid the destruction of virus particles by the immune system and enhance systemic delivery of virus particles, the use of autologous cells as carriers has been explored recently [49]. Mesenchymal stem cells (MSC) mediated delivery of Ad vector overcame the barrier to systemic delivery of Ad vector, and improved intratumoral dissemination of Ad vector [50].

### 5.6 Combination of virotherapy and chemotherapy

Oncolytic Ads are often combined with other therapies. The combination of virotherapy and chemotherapy has shown synergistic response in PCa cells to effectively



kill tumor cells and reduce side effects [51]. The combination of Ad $\Delta\Delta$  (E1B19K- and E1ACR2-deleted) and AdE1A12S enhanced mitoxantrone-induced apoptosis of PCa cells [52]. Onyx-015 was the first oncolytic Ad for clinical trial, and has been evaluated for combined use with topoisomerase II inhibitor etoposide or mitoxantrone [53]. The results showed that tumor growth inhibition was improved when suboptimal doses of chemotherapeutic and Ad vector were combined.

### 5.7 Combination of virotherapy and radiotherapy

Acute single high dose rate (HDR) radiation of PCa cells 24 h before the infection with Ad vector containing PSA enhancer and PB promoter led to significantly enhanced virus replication and cell lysis [54]. In addition, the uptake of radioiodine by injecting Ad carrying the *hNIS* gene linked to *PSMA* (Ad-*PSMApro-hNIS*) had been estimated. The anti-tumor efficacy of radioiodine was significantly improved in C81 cell xenograft model [55]. The results based on 125I nuclide labelled 125I-RSOAdshTERT/PSA could provide new options for the treatment of PCa [56].

### 5.8 Combined modality therapy

Interleukin (IL)-24 exerted inhibitory effects on various cancer cells by enhancing immune regulation and inhibiting tumor growth, angiogenesis and metastasis [57]. Recently, we reported that the combination of Ad vector ZD55-IL-24 with chemotherapy or radiotherapy significantly inhibited the growth of androgen-independent PCa cells and activated the apoptosis of these cancer cells xenografts *in vivo* [58, 59]. These data suggest that the combination of chemotherapy or ionizing radiation and oncolytic Ad vector expressing IL-24 leads to synergistic anti-tumor effect on PCa.

## 6. Conclusions and prospect

Elucidating the molecular mechanism of virus-host interactions of oncolytic adenovirus is essential to the development of better therapy for PCa. The identification of FX,  $\alpha v\beta 6$ , SR-A and SREC-I receptors increases our understanding of tropism and transduction of Ads. A loop in the penton base protein incorporated by a 20-amino acid peptide (A20) reveals a possibility for PCa virotherapy following systemic delivery [60]. Further optimization of Ads with enhanced tropism and transduction is important to achieve high efficacy and specificity to PCa with low toxicity. It is also important to develop synergistic therapy strategies based on oncolytic adenovirus and other treatments such as chemotherapy and radiotherapy.

On the other hand, we should pay attention to the variety in the phenotypes and surface receptors in PCa cells. Although primary cancer cell culture is a golden standard of *in vitro* model, it could not mimic *in vivo* situation of PCa

perfectly. A patient-derived xenograft (PDX) model was developed based on direct engraftment of patient tumor into immunocompromised mice, and it retained most characteristics of primary tumor [61]. The design of novel PCa *in vivo* model will hope realize personalized therapy for PCa patients in near future.

## 7. Author contributions

LM and JW designed the study. CW, FW, ZX collected and analyzed the literatures. RW and JC analyzed the literatures and made the figures. All authors participated in writing the draft and approved the final manuscript.

## 8. Ethics approval and consent to participate

Not applicable.

## 9. Acknowledgment

Not applicable.

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## 11. Conflict of interest

The authors declare no conflict of interest.

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