

Targeting strategies of adenovirus-mediated gene therapy and virotherapy for prostate cancer (Review)

ZHONGLIN CAI^{1*}, HAIDI LV^{1*}, WENJUAN CAO^{1*}, CHUAN ZHOU²,
QIANGZHAO LIU³, HUI LI⁴ and FENGHAI ZHOU¹

¹Department of Urology, Lanzhou General Hospital of Lanzhou Military Command, Lanzhou, Gansu 730050;

²Department of Urology, West China Hospital of Sichuan University, Chengdu, Sichuan 610041; ³Department of Urology, Lanzhou University Second Hospital, Lanzhou, Gansu 730000; ⁴Department of Neurosurgery, Lanzhou General Hospital of Lanzhou Military Command, Lanzhou, Gansu 730050, P.R. China

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Abstract. Prostate cancer (PCa) poses a high risk to older men and it is the second most common type of male malignant tumor in western developed countries. Additionally, there is a lack of effective therapies for PCa at advanced stages. Novel treatment strategies such as adenovirus-mediated gene therapy and virotherapy involve the expression of a specific therapeutic gene to induce death in cancer cells, however, wild-type adenoviruses are also able to infect normal human cells, which leads to undesirable toxicity. Various PCa-targeting strategies in adenovirus-mediated therapy have been developed to improve tumor-targeting effects and human safety. The present review summarizes the relevant knowledge regarding available adenoviruses and PCa-targeting strategies. In addition, future directions in this area are also discussed. In conclusion, although they remain in the early stages of basic research, adenovirus-mediated gene therapy and virotherapy are expected to become important therapies for tumors in the future due to their potential targeting strategies.

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1. Introduction

Adenoviruses. Adenoviruses are linear and non-enveloped double-stranded DNA viruses. The length of genomic DNA is ~36 Kb, and the gene is divided into coding and non-coding regions. The coding region contains five early transcription units (E1A, E1B, E2, E3 and E4), two delayed transcription units (IX and Iva2) and one late transcription unit (L1-L5). A close association exists between E1 (E1A and E1B) and viral replication. E3 is associated with virus immune evasion and is not important for viral replication. Adenoviruses are divided into seven subgroups, A-G, and human adenoviruses encompass 52 types, of which Ad2 and Ad5 are widely employed in adenovirus studies (1,2).

Adenovirus-mediated gene therapy and virotherapy. Gene therapy and virotherapy involve the introduction of therapeutic genes into tumor cells in order to treat tumors. Adenoviruses that mediate anti-tumor therapy include two types of recombinant adenoviruses, which are replication-deficient adenoviruses (RDAd) and conditional replication adenoviruses (CRAd).

The E1 region consists of the E1A gene, E1B-19 kDa (K) gene and E1B-55K gene. These genes regulate viral replication and the gene expression of other early genes. An adenovirus with deletion of E1 is termed a RDAd due to its lack of self-replication (3-9). In adenovirus-mediated gene therapy, the adenovirus is used as a gene vector to induce the expression of therapeutic genes to inhibit tumor growth. However, the lack of a tumor-targeting effect is problematic; RDAd

Correspondence to: Professor Fenghai Zhou, Department of Urology, Lanzhou General Hospital of Lanzhou Military Command, 333 Nanbinhe Road, Qilihe, Lanzhou, Gansu 730050, P.R. China
E-mail: 3073142728@qq.com

*Contributed equally

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may be transduced into normal cells and cause unpredictable cytotoxicity (10). CRAds, also referred to as oncolytic adenoviruses, is one method used in virotherapy and these viruses are capable of self-replication and the delivery of therapeutic genes (11,12). CRAds contain the E1A region that has a key role in viral self-replication. After CRAds infect tumor cells, the virus is able to replicate itself to generate progeny viruses and induce the expression of therapeutic genes. The tumor cells subsequently die and release CRAds and their progeny viruses, which further infect adjacent tumor cells. However, CRAd-infected normal cells survive as CRAd cannot replicate itself inside these cells (13). The following three major strategies are employed to construct these two types of recombinant adenovirus to enhance tumor-targeting: Development of a tumor/tissue-specific promoter/enhancer to induce expression of therapeutic genes and viral replication that is limited to specific tissue or tumor cells (14); modification of adenovirus capsid proteins to construct an adenovirus combined with specific cell surface receptors that efficiently infects specific tissues or tumor cells, with the deletion of partial genes that are essential to adenoviral replication in normal cells but unnecessary for replication in tumor cells (15); and deletion of partial genes that are essential to adenoviral replication in normal cells but unnecessary for replication in tumor cells (16).

2. Development of a prostate-specific promoter/enhancer to induce expression of therapeutic genes and viral replication that is limited to specific tissues or tumor cells

RDAds or CRAds with a prostate-specific promoter or enhancer may exert anti-tumor effects in prostate cancer (PCa) cells only via expression of the therapeutic gene or by oncolysis. Evidence of recombinant adenoviruses with a prostate-specific promoter or enhancer is presented in Table I.

Prostate-specific antigen (PSA). PSA is present in the cytoplasm of prostatic duct epithelial cells and prostate gland cells, and PSA expression has been observed in normal prostate tissues and PCa cells. PSA is the primary biomarker used to monitor PCa. PSA is also employed to screen patients with PCa and monitor the recurrence of PCa following treatment (17-21). CV706 is the first oncolytic adenovirus with the PSA promoter. The PSA promoter drives the expression of E1A and causes the oncolytic adenovirus to replicate in PSA-positive PCa cells and induce oncolysis. However, the ability to self-replicate was low in PSA-negative PCa cells, and its progeny virus production was also low (22,23). In phase I clinical trials, treatment with CV706 was applied to patients with local PCa following radiotherapy, and the results demonstrated a marked decrease in PSA levels and a satisfactory antitumor effect (24). Wang *et al* (25) developed a recombinant adenovirus that expressed β -glucuronidase (β G) under the control of the PSA promoter (Ad/PSAP-GV16- β G). The prodrug DOX-GA3, N-[4-doxorubicin-N-carbonyl (oxymethyl) phenyl] O- β -glucuronyl carbamate, is converted into toxic DOX by β G. The results of an MTT assay indicated that the oncolytic virus induced significant oncolysis in LNCaP PCa cells, however, the same effect was not observed in PSA-negative DU145 PCa cells. In addition, intravenous injection of Ad/PSAP-GV16- β G and treatment with DOX-GA3

efficiently inhibited the growth of LNCaP cell xenograft tumors in nude mice. These results demonstrated the efficacy of the PSA promoter in adenovirus-mediated gene therapy and virotherapy against PSA-producing PCa.

Probasin (PB). PB is a member of the lipocalin superfamily and is a type of ligand transporter. PB is isolated from the nucleus of the dorsal lateral lobe of the rat prostate and is located in the ducts and nucleus of prostate epithelial cells (26,27). As such, PB exhibits tissue specificity, and experiments have demonstrated that a PB promoter may be regulated by androgens and drive the expression of foreign genes in PCa cells *in vitro* and prostate tissue *in vivo* (28). Trujillo *et al* (29) developed a CRAd with PB and Rous sarcoma virus (RSV) promoters that drove the expression of the E1 gene, and NIScDNA-bGH polyA that replaced the E3 region (CRAd Ad5PB_RSV-NIS). *In vitro*, infection of LNCaP PCa cells by the CRAd led to virus replication and cytolysis, and the release of infective viral particles. However, androgen receptor (AR)-negative PC-3 cells (PCa cell line) and Panc-1 cells (pancreatic cancer cell line) infected by the CRAd demonstrated no virus replication or cytolysis. *In vivo*, intratumoral injection with the CRAd and administration of therapeutic ¹³¹iodine in nude mice carrying LNCaP cell xenograft tumors markedly inhibited tumor growth and increased nude mice survival rates. As the RSV promoter induces the expression of therapeutic genes, it may be employed to target cancer cells and normal cells and tissues, and the RSV promoter has a low targeting effect (10). The above results demonstrate that the PB promoter is a prostate-specific promoter. The RDAd (Ad-ARR2PB-Bax) expressed the apoptotic Bcl2-associated X (Bax) gene driven by a PB promoter containing two androgen response elements (ARR). Following infection of LNCaP cells with Ad-ARR2PB-Bax, androgen dihydrotestosterone induced Bax-mediated apoptosis. This antitumor effect of RDAd was also observed in LNCaP xenograft tumors (30). These results indicate that adenoviruses with a PB promoter may be employed to target AR-positive PCa.

Prostate-specific membrane antigen (PSMA). PSMA is a type 2 intrinsic membrane protein on prostatic epithelial cells that is homologous with the serum transferrin receptor. PSMA is primarily expressed in PCa cells and is highly expressed in PCa and during metastasis (31-37). Gao *et al* (38) constructed a recombinant adenovirus that expressed human sodium iodide symporter (hNIS) driven by the PSMA promoter (Ad.PSMApro-hNIS). Compared with the recombinant adenovirus containing a cytomegalovirus (CMV) promoter (Ad.CMV-hNIS), expression of the hNIS gene induced by the PSMA promoter was highly prostate-specific in different LNCaP cell lines, particularly in the androgen-independent C81 LNCaP cell line. The antitumor effect of radioiodine therapy was improved in C81 cell xenografts in nude mice that received PSMA promoter-driven hNIS transfection compared with CMV promoter-driven hNIS transfection. A recombinant adenovirus, combined with the prodrug 5-fluorocytosine, was developed to express the cytosine deaminase (CD) gene driven by a PSMA promoter and enhancer [Ad-PSMA (E-P)-CD]. This treatment caused PSMA-producing PCa cells (LNCaP and CL-1) to regress and efficiently inhibited the growth of

Table I. Evidence for prostate cancer-targeting strategy of tissue/cancer specific promoter/enhancers.

Author, year	Therapeutic type	Promoter	Enhancer	Therapeutic genes	Adenovirus	Combination	Experiment type	Result	(Refs.)
Chen <i>et al</i> , 2001	Virotherapy	NA	PSA	NA	Ad-PSE-E1A	NA	<i>In vitro/vivo</i>	Specific inhibition of tumor/tumor cell growth	(22)
Wang <i>et al</i> , 2016	Gene therapy	PSA	NA	β G	Ad/PSAP-GV 16- β G	Prodrug DOX-GA3	<i>In vitro/vivo</i>	Specific inhibition of tumor/tumor cell growth	(25)
Trujillo <i>et al</i> , 2010	Virotherapy	PB/RSV	NA	NIS	Ad5PB-RSV-NIS	Radioiodine therapy	<i>In vitro/vivo</i>	Specific inhibition of tumor/tumor cell growth	(29)
Andriani <i>et al</i> , 2001	Gene therapy	ARR(2)PB	NA	Bax	Av-ARR(2)PB-Bax	NA	<i>In vitro/vivo</i>	Specific inhibition of androgen-dependent tumor/tumor cell growth	(30)
Gao <i>et al</i> , 2014	Gene therapy	PSMA	NA	NIS	Ad.PSM Apro-hNIS	Radioiodine therapy	<i>In vitro/vivo</i>	Specific inhibition of CRPC/CRPC cell growth	(38)
Zeng <i>et al</i> , 2007	Gene therapy	PSMA	NA	CD	Ad-PSMA (E-P)-CD	Prodrug 5-FC	<i>In vitro/vivo</i>	Specific inhibition of tumor/tumor cell growth	(39)
Fan <i>et al</i> , 2010	Virotherapy	DD3	NA	IL-24	Ad.DD3-E1A-IL-24	NA	<i>In vitro/vivo</i>	Specific inhibition of tumor/tumor cell growth	(43)
Mao <i>et al</i> , 2010	Virotherapy	DD3	NA	SATB1-shRNA	DD3-ZD55-SATB1	NA	<i>In vitro</i>	Specific inhibition of LNCaP cell growth	(44)
Huang <i>et al</i> , 2008	Virotherapy	hTERT	NA	NA	Ad-hTERTp-E1a, OBP301	NA	<i>In vitro/vivo</i>	Specific inhibition of tumor/ tumor cell growth	(59)
Zhang <i>et al</i> , 2006	Gene therapy	hTERT	NA	HSV-TK	Ad-hTERT-HSV-TK	Gangcyclovir	<i>In vitro/vivo</i>	Specific inhibition of tumor/tumor cell growth	(60)
Bhang <i>et al</i> , 2011	Virotherapy	PEG-3	NA	MDA-7/interleukin (IL)-24	Ad.PEG-E1A-MDA-7	NA	<i>In vitro/vivo</i>	Specific inhibition of advanced tumor/ tumor cell growth	(61)
Greco <i>et al</i> , 2010	Virotherapy	PEG-3	NA	MDA-7/interleukin (IL)-24	Ad.PEG-E1A MDA-7	Ultrasound contrast agents, microbubbles	<i>In vitro/vivo</i>	Specific inhibition of advanced tumor/tumor cell growth	(62)
Canales <i>et al</i> , 2006	Virotherapy	BSP	NA	NA	Ad-BSP-E1a	Small molecule inhibitors to telomerase with oligonucleotide-based agents, Taxotere®	<i>In vitro/vivo</i>	Specific inhibition of androgen-independent tumor/tumor cell growth	(72)

Table I. Continued.

Author, year	Therapeutic type	Promoter	Enhancer	Therapeutic genes	Adenovirus	Combination	Experiment type	Result	(Refs.)
Li <i>et al.</i> , 2011	Virotherapy	BSP	NA	NA	Ad-BSP-E1a	NA	<i>In vitro/vivo</i>	Specific inhibition of androgen-independent intraosseous tumor/tumor cell growth	(73)
Yu <i>et al.</i> , 1999	Virotherapy	hK2	hK2, hK2/PSA	NA	Ad5-hK2e-hK2p-E1A, Ad5-PSE-E1A-hK2e-hK2p-E1B	NA	<i>In vitro</i>	Specific inhibition of PSA-positive tumor cell growth	(79)
Koenenman <i>et al.</i> , 2000	Gene therapy	OC	NA	HSV-TK	Ad-OC-HSV-TK	NA	<i>In vitro/vivo</i>	Specific inhibition of androgen-independent metastatic tumor/tumor cells	(85)
Matsubara <i>et al.</i> , 2001	Gene therapy	OC	NA	NA	Ad-OC-HSV-TK	Valacyclovir	I/II clinical trial	One of six patients with hormone-refractory metastatic prostate cancer has exhibited a significant antitumor effect	(87)
Hsieh <i>et al.</i> , 2002	Virotherapy	OC	NA	NA	Ad-OC-E1a	NA	<i>In vitro/vivo</i>	Specific inhibition of androgen-independent metastatic tumor/tumor cells	(88)
Dash <i>et al.</i> , 2010	Virotherapy	OC	NA	NA	Ad-OC-E1a	Vitamin D3	<i>In vitro/vivo</i>	Specific inhibition of androgen-independent metastatic tumor/tumor cells	(89)
Sarkar <i>et al.</i> , 2015	Virotherapy	CCN1/CYR61	NA	MDA-7/IL-24	Ad.tCCN1-E1a	Small molecule E1A-MDA-7 inhibitors of Mcl-1, BI-97D6	<i>In vitro/vivo</i>	Specific inhibition of advanced tumor/tumor cell growth	(92)
Ding <i>et al.</i> , 2012	Virotherapy	DD3	NA	PTEN	Ad.DD3-Δ55-PTEN	NA	<i>In vitro/vivo</i>	Specific inhibition of tumor/tumor cell growth	(115)
Lu <i>et al.</i> , 2013	Virotherapy	PSA, PSMA, MMTV	NA	NA	AdPSAE1, AdPBE1, AdMMTV-E1	NA	<i>In vitro/vivo</i>	AdPSAE1 achieves the most promising oncolysis	(126)

Table I. Continued.

Author, year	Therapeutic type	Promoter	Enhancer	Therapeutic genes	Adenovirus	Combination	Experiment type	Result	(Refs.)
Martiniello-Wilks <i>et al</i> , 2002	Gene therapy	PB	SV40	Purine nucleoside phosphorylase	Ad5-SVPb-PNP	Hormone Ablation therapy	<i>In vitro/vivo</i>	Specific inhibition of androgen-independent tumor/tumor cell growth	(127)
Zhang <i>et al</i> , 2013	Virotherapy	hTERT	NA	Apoptin protein	Ad-hTERTp-E1a-Apoptin	NA	<i>In vitro/vivo</i>	Specific inhibition of tumor/tumor cell growth	(128)
Xie <i>et al</i> , 2001	Gene therapy	hK2	hK2	NA	ADV.hK2-E3/P-EGFP	Androgen Analog (R1881)	<i>In vitro/vivo</i>	Specific inhibition of PSA-positive tumor/tumor cell growth	(129)

NA, not applicable; PSA, prostate-specific antigen; β G, β -glucuronidase; PB, probasin; RSV, Rous sarcoma virus; NIS, sodium iodide symporter; ARR, androgen response element; Bax, Bcl2-associated X; PSMA, prostate-specific membrane antigen; CRPC, castration-resistant prostate cancer; CD, cytosine deaminase; 5-FC, 5-fluorocytosine; DD3, differential display code 3; IL, interleukin; SATB homeobox 1; shRNA, short hairpin RNA; hTERT, human telomerase reverse transcriptase; PEG-3, progression elevated gene-3; MDA, melanoma differentiation-associated protein; BSP, bone sialoprotein; hK2, human kallikrein 2; OC, osteocalcin; HSV-TK, herpes simplex virus thymidine kinase; PTEN, phosphatase and tensin homolog; MMTV, mouse mammary tumor virus; SV40, simian virus 40.

CL-1 xenograft tumors. These results indicate that the PSMA promoter may be an important prostate-specific promoter for adenovirus-mediated treatment of PSMA-positive PCa cells (39).

Prostate cancer gene 3 (PCA3). PCA3 is a type of long non-coding RNA that is one of the PCa-specific markers discovered in recent years. Overexpression of PCA3 occurs in >95% of primary PCa and metastatic cancer specimens, and is not observed in other normal tissues (40-42). Fan *et al* (43) developed two plasmids containing the differential display code (DD)3 of PCA3 promoter and the PSA promoter (pGL3-DD3 and pGL3-PSA, respectively). Luciferase activity demonstrated that the DD3 promoter and the PSA promoter exhibited similar activity in the LNCaP PCa cells. However, the DD3 promoter exhibited ~2-fold higher activity compared with the PSA promoter in DU145 PCa cells. In non-PCa cell lines, the DD3 promoter exhibited a lower activity compared with the PSA promoter. Therefore, the results indicated that the DD3 promoter is more PCa-specific. Furthermore, two oncolytic adenoviruses were developed to express interleukin (IL)-24 driven by the DD3 promoter and the PSA promoter (Ad.DD3-E1A-IL-24 and Ad.PSA-E1A-IL-24, respectively). *In vitro* and *in vivo*, the antitumor effect of Ad.DD3-E1A-IL-24 was higher compared with Ad.PSA-E1A-IL-24. Further experiments demonstrated that the PCa specificity of the DD3 promoter was higher. Mao *et al* (44) reported that the expression of the E1A gene driven by the DD3 promoter of Ad-DD3-E1A occurred in LNCaP PCa cells and not in non-PCa cell lines (BT549 and RWPE2). These results indicate that the DD3 promoter may be useful as a PCa-specific promoter with applications for PCa-targeting by adenovirus-mediated therapy.

Human telomerase reverse transcriptase (hTERT). Telomeres maintain cell chromosome stability and cell activity. Telomere activity is inhibited in normal cells, however, telomerase is reactivated in the majority of human tumor tissues (45-48). High activity of TERT occurs in PCa. However, the activity of TERT is low or absent in normal or benign prostatic hyperplasia tissue (49-52). OBP-301 is an oncolytic virus that contains the hTERT promoter (53-55). OBP-401 is an oncolytic virus that expresses green fluorescent protein (GFP) under control of the hTERT promoter (55-58). When OBP-401 was employed to infect different PCa cell lines (PrEC, PrSC, LNCaP, PC3 and DU145), the expression of GFP occurred in LNCaP, PC3 and DU145 PCa cell lines, but not in PrEC and PrSC normal prostate cell lines. Intratumoral injection with OBP-301 significantly inhibited LNCaP cell xenograft tumors in nude mice. In addition, histological and immunohistochemical analyses demonstrated diffuse oncolysis of tumor cells and the expression of the E1A protein in the tumors (59). Zhang *et al* (60) developed a recombinant adenovirus that expressed the herpes simplex virus-thymidine kinase (HSV-TK) gene driven by the hTERT promoter (Ad-hTERT-HSV-TK). Ad-hTERT-HSV-TK, combined with ganciclovir (GCV), effectively suppressed the growth of LNCaP cell xenograft tumors in nude mice. These results demonstrate that the hTERT promoter is a PCa-specific promoter that may be useful in improving the PCa-targeting effect.

Progression elevated gene-3 (PEG-3). PEG-3 was identified through subtraction hybridization of E11 or E11-NMT cell xenograft tumors during the search for genes involved in malignant transformation and tumor progression. Various trans-acting factors activate PEG-3 in a number of human cancers, including PCa, breast and skin cancer, with limited activity observed in normal tissues. Therefore, PEG-3 exhibits tumor specificity (61-65). Sarkar *et al* (66) constructed an oncolytic adenovirus expressing the melanoma differentiation-associated protein 7 (MDA-7)/IL-24 driven by the PEG-3 promoter (Ad.PEG-E1A-mda-7). Prostatic epithelial cells infected by Ad.PEG-E1A-mda-7 exhibited no expression of E1A and MDA-7, however, expression was observed in LNCaP, DU145 and PC-3 PCa cell lines infected by Ad.PEG-E1A-MDA-7. Ad.PEG-E1A-MDA-7 also markedly inhibited the growth of DU145 cell xenograft tumors *in vitro* and *in vivo* (66). Greco *et al* (62) combined Ad.PEG-E1A-MDA-7 with ultrasound contrast agents (microbubbles) to improve the PCa-targeting effect of the oncolytic adenovirus via ultrasonic guidance. The results demonstrated that microbubble/Ad.MDA-7 complexes markedly reduced the tumor burden in DU145 cell xenograft tumors in nude mice. These results indicate that use of the PEG-3 promoter in the recombinant adenovirus selectively induces the expression of therapeutic genes in PCa.

Bone sialoprotein (BSP). BSP, an acid glycoprotein that is a member of the small integrin-binding, N-linked glycoproteins family, is abundant in the extracellular matrix and is secreted by osteoblasts and osteoclasts (67,68). BSP is associated with the occurrence and development of tumors, and high expression of BSP has been reported in breast cancer, PCa, lung cancer, melanoma and other types of bone metastases (69-71). Canales *et al* (72) developed an oncolytic virus containing the BSP promoter (Ad-BSP-E1a). The oncolytic adenovirus, combined with small molecule antisense oligonucleotide-based inhibitors (GRN163) and Taxotere® (Sanofi S.A., Paris, France), markedly inhibited the growth of the C42B PCa cell line. In addition, Li *et al* (73) reported that the oncolytic adenovirus (Ad-BSP-E1a) inhibited C42B growth and also decreased PSA levels *in vitro*. *In vivo*, the oncolytic adenovirus suppressed the growth of subcutaneous and intraosseous xenograft tumors of the C42B PCa cell line in nude mice (73). These results indicate that the recombinant adenovirus with the BSP promoter has PCa specificity and that CRAds with the BSP promoter have potential for the oncolysis of advanced PCa.

Human kallikrein 2 (hK2). hK2 is a serine protease that is member of the hK family that consists of a highly conserved sequence. hK2 is primarily produced by prostate epithelial cells (74,75) and is also expressed in breast, ovary, testis and other tissues, however, its expression is higher in prostate tissue (75-77). A previous study demonstrated that the hK2 protein was expressed in PSA-negative prostate tumors and in each tumor cell (78). As a result, in addition to PSA, hK2 is considered to be an important marker of PCa. An oncolytic adenovirus mutant that expressed E1A under control of the hK2 promoter/enhancer was referred to as CV763. A study demonstrated that replication of CV763 was notably high in PSA-positive prostate tumor cells, but was attenuated in

PSA-negative and non-prostate tumor cells. CV763 containing the PSA enhancer was referred to as CV764, and exhibited a higher therapeutic index for PSA-positive LNCaP PCa cells (79). The above results indicate that the adenovirus with the hK2 promoter may improve PCa specificity.

Osteocalcin (OC). OC, which is secreted by osteoblasts, is a marker of bone metabolism, and bone is the most common metastatic tissue of advanced PCa. The activity of osteoblasts is closely associated with bone metastasis of tumors. Therefore, OC produced by osteoblasts is also associated with the progression of PCa bone metastasis. Compared with PSA, OC has a high sensitivity and specificity for diagnosing PCa bone metastasis (80-84). Koeneman *et al* (85) constructed an RDAd that expressed HSV-TK driven by the OC promoter (Ad-OC-TK). Ad-OC-TK combined with GCV effectively destroyed PCa cell lines *in vitro* and PCa xenografts *in vivo*, in subcutaneous and bone sites. In phase I clinical trials, patients with local metastasis of PCa were treated with Ad-OC-TK. The results demonstrated that all patients reported an absence of severe side effects, and PCa cell death was observed during treatment (86). Matsubara *et al* (87) reported that an oncolytic adenovirus with the OC promoter effectively inhibited the growth of PCa cell lines (LNCaP, C4-2 and ARCaP). In addition, *in vivo*, this oncolytic adenovirus also markedly suppressed intraosseous xenograft tumors, and PSA levels decreased without a subsequent rebound. Furthermore, combination with vitamin D3 significantly enhanced the antitumor effect of Ad-OC-E1A (88). These results indicate that the recombinant adenovirus containing the OC promoter may be a promising treatment strategy for advanced PCa.

CCN1/CYR61 gene. Elevated expression of the CCN1/CYR61 gene occurs in various cancers, such as advanced PCa, due to oncogenic transformation, and this expression increases with the aggressiveness of the transformed cells (89-91). Sarkar *et al* (92) developed a recombinant adenovirus that expressed MDA-7/IL-24 driven by a truncated (t)CCN1 promoter (Ad.CCN1-CTV-m7). The MDA-7/IL-24 gene under the control of the tCCN1 promoter of Ad.tCCN1-CTV-m7 exhibited high expression in PCa cells. *In vitro*, the Ad.tCCN1-CTV-m7 exerted a dose-dependent killing effect on PCa cells without injury to normal prostatic epithelial cells. *In vivo*, Ad.tCCN1-CTV-m7 significantly suppressed PCa cell xenograft tumors in transgenic Hi-Myc mice when combined with ultrasound-targeted microbubble-destruction. Furthermore, Ad.tCCN1-CTV-m7 combined with small molecule inhibitors of Mcl-1, and BI-97D6, improved apoptosis and tumor growth suppression in Hi-myc mice. These results indicate that the adenovirus with the tCCN1 promoter improved the PCa-targeting effect of the adenovirus and the ability of other treatments to destroy PCa cells.

Combination of promoter and/or enhancer. The combination of a promoter and/or enhancer is a common targeting strategy used to improve PCa specificity of recombinant adenoviruses (Table II). Lee *et al* (93) developed an RDAd with a prostate-specific enhancing sequence (PSES) promoter that consisted of a PSA enhancer and PSMA enhancer (Ad-PSES-luc). Luciferase analysis demonstrated that high

Table II. Evidence for prostate cancer-targeting strategy of combination of promoter and/or enhancer.

Author, year	Therapeutic type	Promoter	Enhancer	Therapeutic genes	Adenovirus	Combination	Experiment type	Results	(Refs.)
Lee <i>et al</i> , 2002	Gene therapy	NA	PSES, PSA/PSMA	NA	Ad-PSES-luc	NA	<i>In vitro/vivo</i>	Demonstrates that PSES exhibits specificity for PSA/PSMA-positive prostate cancer	(93)
Cheng <i>et al</i> , 2006	Virotherapy	TARP	PSES, PSA/PSMA	NA	Ad(I/PPT-E1A)	NA	<i>In vitro/vivo</i>	Specific inhibition of prostate cancer/cells	(95)
Cheng <i>et al</i> , 2004	Gene therapy	TARP	PSES, PSA/PSMA	NA	Ad(I/PPT-Luc)	NA	<i>In vitro/vivo</i>	Demonstrates that PSES exhibits specificity for prostate cancer	(98)
Kraaij <i>et al</i> , 2007	Gene therapy	PB	PSA	NA	Ad5-PSA74-Pb4-EC	NA	<i>In vitro/vivo</i>	Demonstrates specificity for prostate cancer	(99)
Liu <i>et al</i> , 2010	Virotherapy	PB	PSA	NA	Ad5 PSE/PBN E1-AR	Radiation therapy	<i>In vitro/vivo</i>	Specific inhibition of AR-positive prostate cancer/cells	(100)
Li <i>et al</i> , 2005	Virotherapy	NA	PSES, PSA/PSMA	NA	AdE4PSESE1a	NA	<i>In vitro/vivo</i>	Specific inhibition of PSA/PSMA-positive prostate cancer/cells	(130)
Jimenez <i>et al</i> , 2010	Virotherapy	NA	PSES, PSA/PSMA	TRAIL	Ad-E4PSESE1a-TRAIL	NA	<i>In vitro/vivo</i>	Specific inhibition of androgen-independent prostate cancer/cells	(131)

NA, not applicable; PSES, prostate-specific enhancing sequences; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen; TARP, T-cell receptor γ -chain alternate reading frame protein; PB, probasin; AR, androgen receptor; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

expression of the luciferase gene occurred in PSA- and PSMA-expressing PCa cell lines *in vitro* following infection with Ad-PSES-luc. *In vivo*, when Ad-PSES-luc was injected into the prostate, high luciferase activity occurred in the prostate, but not in other tissues. The expression of T-cell receptor γ -chain alternate reading frame protein (TARP) is specific to prostate epithelial cells and PCa cells. The PPT promoter containing the PSA enhancer, the PSMA enhancer and the TARP promoter demonstrates a high specificity for the prostate. The H19 insulator is introduced upstream of the PPT sequence to protect the PPT promoter from transcriptional interference from adenoviral backbone sequences (94-97). Cheng *et al* (98) constructed an adenovirus vector that expressed the luciferase gene under control of the PPT promoter with the H19 insulator [Ad(I/PPT-Luc)]. The I/PPT promoter generated high activities in testosterone-deprived PCa cells and PC-346C PCa cell orthotopic xenograft tumors in nude mice. Cheng *et al* (95) also reported that an oncolytic adenovirus [Ad(I/PPT-E1A)] that infected hormone-dependent and hormone-independent PCa cell lines induced expression of the E1A protein, virus replication and cytolysis *in vitro*, and the growth of LNCaP cell xenograft tumors in nude mice was markedly inhibited *in vivo*. Furthermore, the recombinant adenovirus with the PPT promoter, a two-step transcriptional amplification (TSTA) system, amplified [Ad(PPT/TSTA-Luc)]-enhanced prostate-specific transcriptional activity (97), and the Ad (I/PPT-E1A) with a reintroduced full-length E3 region [Ad (i/PPT-E1A, E3)] improved the cytopathic effect and suppression of PCa growth (96). Kraaij *et al* (99) reported that replication of an adenovirus with the PSA enhancer and the PB promoter (Ad5-PSA74-Pb4-EC) was observed in PCa cells. In addition, an oncolytic adenovirus with the PSA enhancer and the PB promoter (Ad5 PSE/PBN E1-AR), combined with low/high dose-rate radiation, exerted marked adenovirus-mediated PCa cell death (100). Furthermore, Yu *et al* (79) developed an oncolytic adenovirus with the PSA enhancer and the hK2 promoter (CV764). Compared with CV763, CV764 enhanced the inhibitory effects on PCa *in vitro* and *in vivo*. These results demonstrate that a recombinant adenovirus combined with an enhancer and/or promoter produces a higher targeting effect and enhancement of the antitumor effects, which may indicate that adenoviruses combined with other treatments may improve PCa specificity and the suppression of growth.

3. Modification of adenovirus capsid proteins to construct an adenovirus combined with specific cell surface receptors for infection of specific tissue or tumor cells efficiently deletes partial genes that are essential to adenoviral replication in normal cells but are unnecessary for adenoviral replication in tumor cells

Recombinant adenoviruses with modification of adenovirus capsid proteins may enhance the ability to infect PCa cells by binding to the novel receptors on the surface of the cells. Evidence of recombinant adenoviruses with modification of the adenovirus capsid proteins is presented in Table III.

Species C adenoviruses, such as Ad2 and Ad5, infect cells via Coxsackie-adenovirus receptors (CARs) on the cell surface (101). Different levels of CAR expression have been observed in various tumor types and CAR expression is

downregulated in a number of tumors, such as CAR-negative PCa, which results in inefficient Ad-mediated therapeutics (101). Incorporation of an arginine-glycine-aspartic acid (RGD) peptide into the HI loop of the adenovirus fiber knob allows adenoviruses to infect CAR-negative PCa cells via cell-surface integrin $\alpha\beta 3/5$, which is expressed by all PCa cell lines (101). Suzuki *et al* (101) developed an adenovirus mutant with an RGD-fiber modification (Ad5- Δ 24RGD). Compared with an adenovirus mutant without the RGD-fiber modification (Ad5- Δ 24), Ad5- Δ 24RGD exhibited a higher infection ability and an anti-PCa effect. A number of studies involving recombinant adenoviruses with RGD-fiber modification further confirmed that the RGD-modified adenovirus may enhance the PCa-targeting effects *in vitro* and *in vivo* (102-105).

The generation of chimeric adenoviruses, in which one adenovirus fiber knob is replaced with a different adenovirus fiber knob, may alter the orientation of the adenovirus and enhance transduction targeting to improve the tumor cell infection efficiency. Azab *et al* (106) constructed a recombinant adenovirus in which the fiber knob was replaced with an Ad.3 fiber knob, and this construct expressed the MDA-7/IL-24 gene (Ad.5/3-CTV). Compared with Ad.5-CTV, Ad.5/3-CTV exhibited a higher efficiency in inhibiting the viability of low-CAR human PCa cells *in vitro*, and also potentially suppressed low-CAR PCa cell xenograft tumors *in vivo*. It has been reported that the Ad.3 receptor is highly expressed in tumor cells (107). Ad.5/3 infected the tumor cells via the Ad.3 receptor instead of CAR, and, therefore, it was able to infect tumor cells with low or no expression of CAR (107-109). Systemic treatment with Ad.5 is associated with serious hepatotoxicity and systemic toxicity (110). Xu *et al* (110) developed a chimeric oncolytic adenovirus that expressed soluble transforming growth factor β receptor II-Fc fusion protein (sT β RFc), the chimeric oncolytic adenovirus in which seven hypervariable regions of Ad.5 were substituted with the corresponding sequence of Ad48 (mHAd.sT β RFc). *In vivo*, mHAd.sT β RFc retained an inhibitory effect on PC-3 PCa bone metastases in nude mice, and also reduced the hepatotoxicity and systemic toxicity to indirectly improve the tumor-targeting effect. Serotype 35 adenoviruses infect cells through cell surface CD46 receptors, which are widely expressed on normal and cancer cells (111). Kim *et al* (111) constructed a novel chimeric recombinant adenovirus expressing monomeric red fluorescence protein (mRFP)/modified HSV-TK (ttk) (Ad5/35PSES.mRFP/ttk), which was driven by PSES and featured the serotype 35 fiber knob on the serotype 5 backbone. This chimera improved the cell infection efficiency, and the PSES enhanced the PCa-targeting effect. *In vitro*, replication assays demonstrated that Ad5/35PSES.mRFP/ttk replicated in PSES-positive PCa cells (LNCaP and CWR22rv) but not in PSES-negative PCa cells (DU145 and PC3). Evaluation of the cytotoxic activity demonstrated that Ad5/35PSES.mRFP/ttk killed LNCaP and CWR22rv cells more effectively. In addition, the chimeric oncolytic adenovirus Ad5/35E1aPSESE4 also effectively killed PSA/PSMA-positive PCa cells in the peripheral circulation (112).

4. Deletion of partial genes that are essential to adenoviral replication in normal cells but are unnecessary for

Table III. Evidence for prostate cancer-targeting strategy of modification of adenovirus capsid proteins.

Author, year	Therapeutic type	Method of modification of the fiber knob	Therapeutic genes	Adenovirus	Combination	Experiment type	Result	(Refs.)
Suzuki <i>et al</i> , 2001	Virotherapy	Incorporation of an RGD peptide into the HI loop of the fiber knob	NA	Ad5-Δ24RGD	NA	<i>In vitro/vivo</i>	Specific inhibition of prostate cancer/cells	(101)
Cody <i>et al</i> , 2013	Virotherapy	Incorporation of an RGD peptide into the HI loop of the fiber knob	OPG	Ad5-Δ24-sOPG-Fc-RGD	NA	<i>In vitro/vivo</i>	Specific inhibition of progression of prostate cancer bone metastases	(102)
Shen <i>et al</i> , 2016	Virotherapy	Incorporation of an RGD peptide into the HI loop of the fiber knob	NA	AxdAdB3-F/RGD	NA	<i>In vitro/vivo</i>	Specific inhibition of CAR-deficient prostate cancer/cells	(105)
Azab <i>et al</i> , 2014	Virotherapy	Replacing the Ad the Ad.3 fiber knob	MDA-7/IL-24	Ad.5/3-PEG-E1A-MDA-7/IL-24, Ad.5/3-CTV	NA	<i>In vitro/vivo</i>	Specific inhibition of advanced prostate cancer/cells	(106)
Hakkarainen <i>et al</i> , 2009	Virotherapy	Replacing the Ad.5 fiber knob with the Ad.3 fiber knob	NIS	Ad5/3-Δ24-hNIS	Radioiodine therapy	<i>In vitro/vivo</i>	Specific inhibition of prostate cancer/cells	(108)
Xu <i>et al</i> , 2014	Virotherapy	Replacing seven hypervariable regions of Ad5 hexon with the Ad48	sTGβRIIFc	Ad5/48.sTβRFc, mHAd.sTβRFc	NA	<i>In vitro/vivo</i>	Specific inhibition of progression of prostate cancer bone metastases	(110)
Kim <i>et al</i> , 2013	Virotherapy	Insertion of serotype 35 fiber knob into the serotype 5 backbone	sFLT3L ligand and mRFP/ttk	Ad5/35PSES, mRFP/ttk	NA	<i>In vitro</i>	Specific inhibition of PSES-positive prostate cancer cells	(111)
Hwang <i>et al</i> , 2016	Virotherapy	Insertion of serotype 35 fiber knob into the serotype 5 backbone	NA	Ad5/35E1aPSESE4	NA	<i>In vitro/vivo</i>	Specific inhibition of PSES-positive circulating prostate tumor cells	(112)

RGD, arginine-glycine-aspartic acid; NA, not applicable; OPG, osteoprotegerin; CAR, Cocksackie-adenovirus receptor; MDA, melanoma differentiation-associated protein; IL, interleukin; NIS, sodium iodide symporter; sTGβRIIFc, soluble transforming growth factor β receptor II-Fc fusion protein; sFLT3L, soluble fms-related tyrosine kinase 3 ligand; mRFP, monomeric red fluorescence protein; ttk, modified herpes simplex virus thymidine kinase; PSES, PSES, prostate-specific enhancing sequences.

adenoviral replication in tumor cells

Binding of E1B-55K protein to the p53 gene inhibits p53-mediated normal cell apoptosis, therefore, oncolytic adenoviruses with the E1B-55K gene are able to survive in normal cells (113-115). Oncolytic adenoviruses with deletion of the E1B-55K gene have the ability to survive in tumor cells in which no apoptosis occurs due to p53 gene mutations or deficiency. However, oncolytic adenoviruses with deletion of the E1B-55K gene cannot survive in normal cells due to p53-mediated apoptosis (113). Mao *et al* (114) developed an oncolytic adenovirus with deletion of the E1B-55K gene, the oncolytic adenovirus that expresses short hairpin RNA targeting SATB1 (SATB1; ZD55-SATB1). ZD55-SATB1 markedly inhibited the viability and invasion of PCa cell lines DU145 and LNCaP, and suppressed PCa growth and metastasis in xenograft nude mice. Ding *et al* (115) reported that an oncolytic adenovirus mutant with the DD3 promoter and deletion of the E1B-55K gene, termed Ad.DD3.Δ55-PTEN, expressed phosphatase and tensin homolog (PTEN) to induce PCa cell apoptosis and inhibit the growth of xenograft tumors, however, Ad.DD3.Δ55-PTEN had no death-inducing effects in non-PCa cell lines.

The E1A conserved region 2 (E1A-CR2) normally binds to host cell retinoblastoma (Rb) protein and releases transcription factor E2F, enabling S-phase entry and viral DNA replication. Oncolytic adenovirus E1A-CR2 (Rb-family binding site) mutants do not bind to Rb protein to induce normal cells to enter S phase and, therefore, are unable to efficiently replicate in quiescent normal tissues. However, oncolytic adenovirus E1A-CR2 mutants are able to replicate in tumor cells with Rb gene mutations as tumor cell growth is not solely dependent on Rb protein (14). In addition, adenovirus E1A-CR2 mutants combined with cytotoxic drugs (116) or radiotherapy (108) significantly enhance the inhibitory effect on castration-resistant PCa. A novel oncolytic adenovirus mutant with deletion of E1A-CR2 and E1B-19K, referred to as AdΔCR2Δ19K, demonstrated high cytotoxic effects in PCa, pancreatic cancer and lung cancer, and the replication ability of AdΔCR2Δ19K in tumor cells was similar to that of the wild-type virus (117). Radhakrishnan *et al* (116) constructed an oncolytic adenovirus mutant with deletion of E1A-CR2 and E3B (dl922-947). Compared with dl312 (ΔE1A and ΔE3B), dl1520 (ΔE1B-55K and ΔE3B) and Ad5 (wild-type), dl922-947 exhibited the highest antitumor effect in hormone-independent PCa *in vitro* and *in vivo*. The combination of dl922-947 with low doses of mitoxantrone or docetaxel enhanced the efficacy. Furthermore, Satoh *et al* (118) developed a double-mutated adenovirus with a mutation in E1A-CR2 and deletion of E1B-55K (AxdAdB-3). *In vitro*, AxdAdB-3 exhibited a potential cytopathic effect in different PCa cell lines and demonstrated no cytotoxicity in PrEC and PrSC normal prostate cell lines. *In vivo*, AxdAdB-3 markedly inhibited the growth of PCa cell xenograft tumors in nude mice and improved survival.

Adenovirus mutants have substantial effects on the inhibition of the growth of PCa and a number of mutations in the E1 region of the adenovirus are associated with these effects. A list of adenovirus mutants is presented in Table IV.

5. Clinical research on adenovirus-mediated gene therapy

and virotherapy for prostate cancer

Currently, viral gene therapy is an area of increasing interest in the field of tumor therapy. Adenovirus-mediated gene therapy and virotherapy are among the most common research areas in viral gene therapy. As these therapies have demonstrated satisfactory anti-PCa effects in basic experiments, clinical trials have been performed. DeWeese *et al* (119) performed a phase I clinical trial in which 20 patients with PCa who had relapsed following radiotherapy were treated with CRAD CV706. The clinical results demonstrated a satisfactory treatment effect on PCa without the presence of severe side effects. In addition, Freytag *et al* (120) constructed an oncolytic virus (ZD55-CD/TKrep) with deletion of E1B-55K and expression of the suicide gene CD/TKrep, which was employed to salvage therapy for 16 patients with PCa who had relapsed following radiotherapy. The clinical results indicated good safety and efficacy. A total of 16 patients were followed for 5 years and the survival rate was 88% (14/16 patients). Furthermore, Freytag *et al* (121) used an oncolytic virus (ZD55-CD/TKrep) combined with external radiotherapy to treat 15 patients with high-risk PCa. The results demonstrated that the effect of combined therapy was higher compared with radiotherapy alone, however, contradictory clinical effects have also been reported regarding PCa in clinical trials. Small *et al* (122) conducted a phase I trial of intravenous CG7870 to treat hormone-refractory metastatic PCa. The results indicated a poor treatment effect, and patients with decreased serum PSA levels accounted for only 5/23 patients with PCa. However, no obvious side effects were observed in the 23 patients. Although the majority of clinical trials concerning adenovirus-mediated gene therapy and virotherapy have demonstrated good antitumor effects, biosafety issues arise with adenovirus treatments, particularly tumor-targeting treatments, which limits clinical applications. Consequently, clinical trials involving adenovirus treatments have been stalled in phase I clinical trials. Currently, only one type of oncolytic adenovirus, H101 with deletion of E1B-55K, has been approved for use in patients with advanced tumors, and this approval is only in China.

6. Future directions

Although adenoviruses constructed by different targeting strategies have demonstrated satisfactory targeting effects in the treatment of PCa, each targeting strategy is associated with certain limitations. The combined use of multiple targeting strategies to enhance the adenovirus targeting effect is one promising direction. Currently, several experiments with adenoviruses constructed using multiple targeting strategies have demonstrated that the adenoviruses markedly improve targeting and antitumor effects, including AxdAdB3-F/RGD (105) with RGD-fiber modification and the E1A/E1B double mutation, Ad5/3-Δ24-hNIS (108) with the hybrid Ad5/3 fiber and 24-bp deletion in the E1A-CR2, and DD3-ZD55-SATB1 (114) with the DD3 promoter and E1D-55 K deletion, among others. Therefore, the joint use of targeting strategies is an important direction towards enhanced tumor targeting. A list of the adenoviruses constructed using multiple targeting strategies is presented in Table V.

Table IV. Evidence for prostate cancer-targeting strategies of adenoviral mutants.

Author, year	Therapeutic type	Mutational pattern	Therapeutic genes	Adenovirus	Combination	Experiment type	Result	(Refs.)
Cody <i>et al</i> , 2013	Virotherapy	A 24-bp deletion in the E1A conserved region 2,Δ24	OPG	Ad5-Δ24-sOPG-Fc-RGD	NA	<i>In vitro/vivo</i>	Specific inhibition of progression of prostate cancer bone metastases	(102)
Hakkarainen <i>et al</i> , 2009	Virotherapy	A 24-bp deletion in the E1A conserved region 2,Δ24	NIS	Ad5/β-Δ24-hNIS	Radioiodine therapy	<i>In vitro/vivo</i>	Specific inhibition of prostate cancer/ cells	(108)
Mao <i>et al</i> , 2015	Virotherapy	Deletion of E1B-55K	shRNA targeting SATB1	ZD55-SATB1	NA	<i>In vitro/vivo</i>	Specific inhibition of prostate cancer growth and metastasis	(114)
Ding <i>et al</i> , 2012	Virotherapy	Deletion of E1B-55K	PTEN	Ad.DD3.D55-PTEN	NA	<i>In vitro/vivo</i>	Specific inhibition of tumor/tumor cell growth	(115)
Radhakrishnan <i>et al</i> , 2010	Virotherapy	Deletion of E3B/a 24-bp deletion in the E1A conserved region 2,Δ24	NA	Ad-Δ55KΔE3B	Mitoxantrone/docetaxel	<i>In vitro/vivo</i>	Specific inhibition of androgen-independent prostate cancer/cells	(116)
Oberg <i>et al</i> , 2010	Virotherapy	Deletion of E1B-19K/a 24-bp deletion in the E1A conserved region 2,Δ24	NA	Ad-ΔCR2Δ19K	Mitoxantrone/docetaxel	<i>In vitro/vivo</i>	Specific inhibition of androgen-independent prostate cancer/cells	(117)
Satoh <i>et al</i> , 2007	Virotherapy	Deletion of E1B-55K/a 24-bp deletion in the E1A conserved region 2,Δ24	NA	AxAdAdB-3	NA	<i>In vitro/vivo</i>	Specific inhibition of androgen-independent prostate cancer/cells	(118)

OPG, osteoprotegerin; NA, not applicable; NIS, sodium iodide symporter; -55/19K, -55/19 kDa; shRNA, short hairpin RNA; SATB homeobox 1; PTEN, phosphatase and tensin homolog.

Table V. Evidence for the combined use of prostate cancer-targeting strategies.

Author, year	Therapeutic type	Method of modification of the fiber knob	Mutational pattern	Promoter	Enhancer	Therapeutic genes	Adenovirus	Combination	Experiment type	Result	(Refs.)
Suzuki <i>et al</i> , 2001	Virotherapy	Incorporation of an RGD peptide into the HI loop of the fiber knob	A 24-bp deletion in the E1A conserved region 2, Δ24	NA	NA	NA	Ad5-Δ24RGD	NA	<i>In vitro/vivo</i>	Specific inhibition of prostate cancer/cells	(101)
Shen <i>et al</i> , 2016	Virotherapy	Incorporation of an RGD peptide into the HI loop of the fiber knob	SXGXE (STGHE) mutation in E1A/ Deletion of E1B-55K	NA	NA	NA	AxdAdB3-F/RGD	NA	<i>In vitro/vivo</i>	Specific inhibition of CAR-deficient prostate cancer/cells	(105)
Azab <i>et al</i> , 2014	Virotherapy	Replacing the Ad.5 fiber knob with the Ad.3 fiber knob	NA	PEG	NA	MDA-7/ IL-24	Ad.5/3-PEG-E1A-MDA-7/ IL-24, Ad.5/3-CTV	NA	<i>In vitro/vivo</i>	Specific inhibition of advanced prostate cancer/cells	(106)
Hakkarainen <i>et al</i> , 2009	Virotherapy	Replacing the Ad.5 fiber knob with the Ad.3 fiber knob	A 24-bp deletion in the E1A conserved region 2, Δ24	NA	NA	NIS	Ad5/3-Δ24-hNIS	Radioiodine therapy	<i>In vitro/vivo</i>	Specific inhibition of prostate cancer/cells	(108)
Hwang <i>et al</i> , 2016	Virotherapy	Insertion of serotype 35 fiber knob into the serotype 5 backbone	NA	NA	PSES, PSA/ PSMA	NA	Ad5/35E1a PSESE4	NA	<i>In vitro/vivo</i>	Specific inhibition of PSES-positive circulating prostate tumor cells	(112)
Ding <i>et al</i> , 2012	Virotherapy	NA	Deletion of E1B-55K	DD3	NA	PTEN	Ad.DD3, D55-PTEN	NA	<i>In vitro/vivo</i>	Specific inhibition of tumor/tumor cell growth	(115)

RGD, arginine-glycine-aspartic acid; NA, not applicable; -55 K, -55 kDa; CAR, Coxsackie virus adenovirus receptor; PEG, progression elevated gene; MDA, melanoma differentiation-associated protein; IL, interleukin; NIS, sodium iodide symporter; PSES, prostate-specific enhancing sequences; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen; DD3, differential display code 3; PTEN, phosphatase and tensin.

Another promising direction for tumor-targeting strategies takes advantage of the host immune system. The immune system is a potent defensive capability that protects the body from disease, including tumor development and progression. However, certain tumors exhibit host immune tolerance. Adenoviruses armed with cytokines or inhibitors are able to weaken tumor-associated immune checkpoint inhibition, and the host immune tolerance of the tumor may also be reduced (123-125). Following lysis of tumor cells infected by the adenovirus, tumor antigen exposure activates host tumor immunity to induce lysis of metastatic lesions (123). Several adenoviruses have been developed to trigger these oncolytic immunotherapeutic effects, and the results have been satisfactory in certain tumors. Adenovirus mutant Ad5Δ24/3-RGD-GM-CSF, with expression of granulocyte macrophage-colony-stimulating factor (GM-CSF), exhibits potent antitumor effects in PCa. This construct induced tumor cell death and activated T-cells in response to antigen presentation by exposure of the tumor antigen. The mounted immune response of the injected tumor improved immune recognition to attenuate the growth of distant metastases in PCa (123). Pexa-Vec, which is an oncolytic poxvirus expressing GM-CSF, markedly inhibited tumor progression by inducing host tumor immunity (124). A HSV-1 mutant, termed T-VEC, also expressed GM-CSF to activate antitumor immunity and induced regression of non-injected distal lesions in advanced melanoma (125). Although Pexa-Vec and T-VEC have not yet been used to treat PCa, we hypothesize that treatment of PCa with adenoviruses constructed using an identical strategy may achieve beneficial responses. Adenoviruses armed with cytokines or inhibitors are the most promising strategy for the targeted treatment of early- and late-stage PCa.

7. Conclusion

In conclusion, the tumor-targeting effect is the key point regarding adenovirus-mediated gene therapy and virotherapy. Targeting strategies have been increasingly developed in basic research, however, various limitations remain. Therefore, further research concerning targeting strategies is required to improve the safety of these therapies in the human body and to maximize the net benefit of adenovirus-mediated gene therapy and virotherapy.

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