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Development of Oncolytic Adenoviruses for the Management of Prostate Cancer

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Abstract

Prostate cancer (PCa) is the fifth most common cause of cancer-related deaths in men globally. Androgen receptor (AR) signalling plays a vital role in initiation and progression and antiandrogens are standard of care first-line therapeutics. However, resistance frequently develops resulting in metastatic castration-resistant prostate cancer (mCRPC). Management of CRPC is currently chemotherapy and/or radiotherapy but is mostly palliative due to rapid development of resistance. The need for novel approaches to eliminate mCRPC is compelling; a promising option is replication-selective (oncolytic) adenoviruses with demonstrated efficacy in preclinical models of multidrug-resistant PCa. The safety of various viral mutants has been confirmed in numerous clinical trials with minimal toxicity in patients. Importantly, oncolytic adenoviruses synergise with the current standard of care for mCRPC even in treatment-resistant cells. In early phase I–II clinical trials, promising efficacy in patients with localised PCa was reported after intratumoural administration, and phase III trials are underway. To enable systemic delivery, for targeting of mCRPC, further developments are necessary because of the short half-life of the adenoviral mutants in human blood. Current progress in preventing the high-affinity binding of adenovirus to erythrocytes, hepatocyte uptake, and elimination by hepatic Kupffer cells will be described.

Keywords: prostate cancer, oncolytic adenoviruses, androgen, treatment resistance, viral modifications

1. Introduction

The current treatment approaches for prostate cancer (PCa) are successfully managing local disease with a reported 5-year survival rate of 100% [1]. At this stage, the treatment options

are surgery (radical prostatectomy), radiation therapy, and androgen deprivation therapy (ADT), which includes castration, androgen receptor (AR) inhibition, and combined therapies. Castration is classified as either surgical (orchiectomy) or medical, for example, administration of luteinizing hormone-releasing hormone (LHRH) agonists or antagonists. Current use of AR inhibitors includes the nonsteroidal antiandrogens (NSAA) nilutamide, flutamide, and bicalutamide, which have demonstrated better tolerability than earlier steroidal antiandrogens such as cyproterone acetate. Combined androgen blockade (CAB) refers to the use of castration and AR antagonists combined [2]. In contrast, late-stage hormone-independent metastatic PCa has a 5-year survival rate of only 29% because of the development of resistance to all current therapeutics including cytotoxic drugs [1]. There is an unmet medical need for management of late-stage PCa. Efforts to improve the survival of patients with metastatic PCa have led to the development of novel therapeutics with the majority of agents targeting the androgen pathway, for example, the NSAA ARN-509 (Aragon Pharmaceuticals) and the androgen synthesis inhibitor abiraterone [3, 4]. However, only limited survival benefits and development of resistance have been observed with the new agents. A promising novel class of therapeutics that act through entirely different mechanisms than traditional cytotoxic and targeted drugs is oncolytic viruses. Currently, no oncolytic virus has been approved for treatment of PCa, although numerous phase I–II trials have been completed with promising outcomes and phase III trials are underway [5]. The most promising preclinical and clinical efficacy has been reported for various PCa-selective replicating adenoviral mutants that lyse cancer cells and leave normal cells unharmed and, in addition, resensitise drug-resistant cancer cells to chemotherapeutics [6–8].

1.1. Oncolytic viruses and prostate cancer

Gene therapy with oncolytic viruses is currently one of the most promising approaches for cancer elimination based on both preclinical data and results from numerous clinical trials. While classical gene therapy uses nonreplicating viruses as vectors to deliver transgenes to cancer cells, oncolytic virus therapy employs the lytic properties of replicating viruses to lyse cancer cells in addition to expression of cytotoxic transgenes to enhance efficacy and spread within the tumours [9]. Oncolytic viruses are engineered to replicate selectively in tumour cells and are most often genetically modified to selectively infect, propagate, and kill cancer cells without affecting normal cells [9, 10].

The concept of using replicating viruses in cancer treatment is not new; over a century ago, it was noted that tumours regressed in patients after naturally occurring systemic viral infections [10, 11]. During 1950–1980, several clinical trials were carried out to assess the ability of wild-type viruses to eliminate cancer, including the yellow fever, hepatitis, adenoviruses, and West Nile fever viruses [12]. However, the outcomes were not conclusive due to failure of infection control and spread to both healthy and malignant cells with poor patient outcomes. At present, it is well known that most cancer cells have impaired innate immune responses with decreased protection for viral infection, for example, altered interferon activity, resulting in enhanced viral replication in cancer cells compared to normal cells [13]. For this reason, the main challenges with viral therapies today are to prevent replication in normal cells rather than increase replication in tumour cells (**Figure 1**).
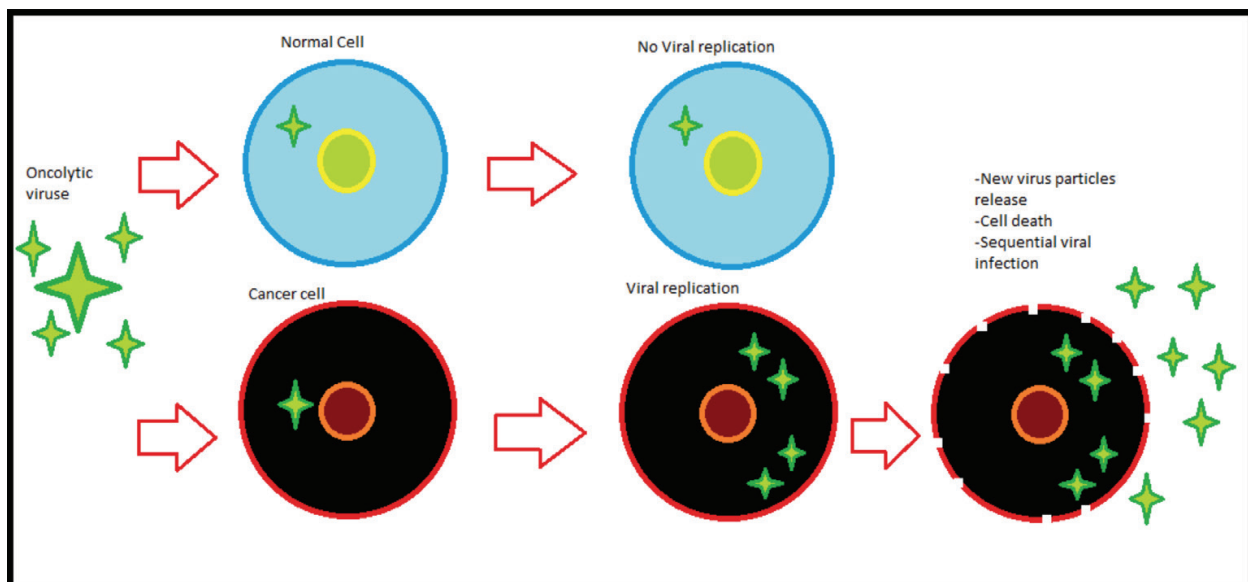
Figure 1. Selective replication and killing of cancer cells. Oncolytic viruses are engineered to replicate only in cancer cells by deleting viral genes that are essential for viral propagation in normal cells and are complemented in cancer cells by the altered gene expression in tumours. Alternatively, tumour-specific promoters are inserted in the viral genome to drive viral gene expression and propagation in cancer cells only. While oncolytic viruses may infect normal healthy cells viral propagation cannot proceed. In contrast, in cancer cells, viral infection leads to potent viral gene expression, genome amplification and virion assembly followed by virus-induced cell lysis and spread to surrounding tumour tissue.

In order to generate viruses that are cancer-selective, the functions of the viral gene-products must be fully understood to enable selection of the appropriate genes for engineering. One of the first oncolytic viral mutants for targeting of cancer cells was generated in 1991, by Martuza et al. [14]. The virus, a herpes simplex virus type 1 (HSVI) deleted in the thymidine kinase (TK) gene, which is essential for replication in normal cells, demonstrated good results in killing human glioblastoma cells both *in vitro* and in models *in vivo* [14]. A modified adenovirus, Oncorine or H101 (based on Onyx-015; [15]), was the first genetically engineered oncolytic virus to be approved for cancer therapy [16]. In 2005, the Chinese FDA granted market approval for Oncorine as an anticancer agent for hepatic and head and neck cancers. The phase III clinical trial that led to the approval assessed the benefits of adding Oncorine to cisplatin-based chemotherapy in the treatment of advanced head and neck squamous cell tumours by intratumoural administration. The objective response rates for the combination treated group were reported at 79%, while cisplatin alone resulted in 40% regression of injected tumours. However, no overall survival benefits were observed. The only other oncolytic virus on the market is Imlygic or T-VEC (Amgen), an HSVI mutant expressing granulocyte-macrophage colony-stimulating factor (GM-CSF) [17]. Imlygic was licenced in 2015 for melanoma by the FDA and approved in Europe in 2016 and in Australia in 2015. Currently, there are several engineered oncolytic viruses undergoing phase III clinical trials including a poxvirus (Pexa-Vec; JX-594; Transgene) for advanced hepatocellular carcinoma and the adenovirus mutant (CG0070; Cold Genesys) for bladder cancer [10]. During 2001–2014, six clinical trials investigating oncolytic virus therapy for recurrent localised prostate cancer were reported; four of them evaluated adenovirus-based therapies and two reovirus-based therapies, summarised in **Table 1** [18–23].

Currently, several phase I–II trials are ongoing using modified oncolytic adenoviruses. Although the development and use of oncolytic viruses have resulted in promising pre-clinical and clinical results, several challenges remain such as premature elimination of virus by the host immune system, viral pathogenic mechanisms, and failure to target all lesions at metastatic sites.

Virus	Genetic modifications	Phase/number of patients	Toxicity/route of administration	Outcomes
CV706	E1A expression controlled by PSA-promoter-enhancer	Phase I 20	Low Intraprostatic	65% of the patients had >30% serum PSA reduction and 25% had >50% serum PSA reduction [18]
CG7870	E1A expression controlled by rat-probasin-promoter E1B expression controlled by PSA-promoter-enhancer	Phase I 23	Grade 1 or 2, 13% grade 3 Intravenous	27% without PSA progression at 6 months, median time to PSA progression 60 days [19]
Ad5-CD/TK*rep*	E1B55K-deleted, armed with suicide genes (herpes simplex type 1 thymidine kinase cytosine deaminase)	Phase I 16	Grade 1 or 2 Intraprostatic	44% had ≥25% decrease in serum PSA level; 19% had ≥50% decrease in serum PSA level [20]
Ad5-CD/TK*rep*	E1B55K-deleted, armed with suicide genes (herpes simplex type 1 thymidine kinase cytosine deaminase)	Phase II 44	Grade 1 or 2 Intraprostatic	≥2 years after treatment, reduced biopsy positivity overall from actual biopsies (42%) and intention-to-treat (34%), and men with <50% biopsy positivity 60% [21]
Ad5-yCD/ mutTK(SR39) rep-hNIS	E1B55K-deleted, hNIS as a reporter gene to monitor virus replication and efficacy	Phase I	Ongoing	Ongoing [59]
Ad5-yCD/ mutTK(SR39) rep-hIL12	E1B-55K-deleted, armed with IL12	Phase I	Ongoing	Ongoing [60]
Reolysin®	None (wild type)	Phase I 5	Grade 1 and 2 Intravenous	51% decrease in PSA level in one patient with prostate cancer [22]
Reolysin®	None (wild type)	Phase I 4	Low, dose-limiting grade 4 neutropenia in one patient Intravenous	30% decrease in PSA level [23]

Table 1. Published prostate cancer clinical trials with oncolytic viruses.

2. Development of oncolytic adenoviruses

The most promising oncolytic viruses are genetically modified adenoviruses. Adenoviral mutants are continuously being developed to further improve on selectivity and efficacy. Adenovirus was discovered in the 1950s when it was isolated from adenoid tissues [24]. The linear double-stranded DNA genome, 30–38 kb, is enclosed in a protein capsid, forming 70–100 nm virion particles with icosahedral symmetry. There are more than 50 subtypes of the human adenovirus family that infect a broad range of host tissues often causing acute mild disease including respiratory infections, epidemic conjunctivitis, and infantile gastroenteritis [25, 26]. Despite the ability of certain subtypes to induce cancer in rodent models and transform cultured cells, there is no evidence to date that adenoviruses cause cancer in humans [27]. Currently, most clinical and preclinical studies have employed adenoviral mutants generated from type 5 (Ad5) because of its proven safety record and known functions.

2.1. Structure of adenoviruses

The viral capsid consists of 240 hexon and 12 penton proteins with fibre proteins projecting from the pentons and several small proteins that aid in stabilising the icosahedral structure [28]. The DNA containing core harbours additional proteins, the major polypeptide V and VII, a minor arginine-rich protein μ, which is covalently attached to the 5′-ends of the DNA, and the terminal proteins that bind to the DNA ends to act as primers for DNA replication. The viral DNA is wrapped around polypeptide VII similar to human DNA and histone proteins, and polypeptide V binds to the pentons to serve as a bridge between the core and the capsid.

The first adenoviral genome to be sequenced was from subtype 2 (Ad2), composed of 35,937 base pairs (bp) [29]. Since then, the majority of subtypes have been sequenced and found to have similar genome organisation and functional gene products as Ad2, including Ad5. The genome is divided into early expressed units (E1A, E1B, E2A, E2B, E3, and E4), delayed early units (IX and IVa2), and late units (L1–L5). The early units are the first to be expressed and encode proteins responsible for initiating transcription of other viral genes and for changing the intracellular environment to support viral production [30, 31]. The E1A proteins are required for productive infection and induce S-phase, cellular DNA synthesis and activate viral gene expression. The E2 proteins code for the viral DNA polymerase, which is essential for viral genome amplification. The E3 and E4 proteins are not essential for viral replication but prevent premature cell death of infected cells in response to the host immune defence and inhibit the DNA-damage repair, respectively. The late regions encode the viral structural proteins after viral genome amplification to encapsulate newly synthesised viral DNA. The VA RNA I and II reduce stimulation of the interferon response, delay cellular microRNA processing, and control the expression of host genes. Both ends of the genome contain the 100 bp inverted terminal repeats (ITRs), which serve as the origin of replication, and the viral packaging sequence (~200 bp), which is located next to the left ITR.

The viral particle enters the host cell through receptor-mediated endocytosis due to the interactions between the viral fibre and coxsackievirus and adenovirus receptor (CAR), and

between pentons and integrins, mainly $\alpha v\beta 3$ and $\alpha v\beta 5$. The viral DNA is released in the endosome and transported to the nucleus where E1A is expressed constitutively, initiating expression of other early genes and hijacking the host cell DNA-synthesis machinery [31]. After complete viral genome amplification and assembly of new particles, the host cell is lysed and viruses spread and infect surrounding cells [32].

2.2. Anticancer activity of adenoviruses

Clinical evaluation of adenoviruses as oncolytic therapeutics started shortly after the discovery in the 1950s [11, 33]. The small genome is easy to engineer with known functions of the majority of the gene products, the genome is not integrated into the host cell DNA, the clinical safety profile is excellent with only flu-like side effects, and the natural tropism to epithelial cells renders adenocarcinomas, including PCa, excellent targets. Clinical developments have been limited to some extent because of the frequent downregulation of the native adenovirus receptor CAR in many tumours and the presence of preexisting antiadenoviral antibodies. A majority of the population has been infected with adenovirus at some point [27]. Several approaches have been explored to solve these limitations and are described below.

2.2.1. Viral fibre modifications

A promising and now common strategy that has been assessed in both preclinical and clinical studies is the modification of various regions of the viral capsid to enhance the affinity and therapeutic effects in CAR-negative cancer cell lines [27]. Several teams have evaluated fibre modifications that incorporate a partial peptide sequence from fibronectin, containing an arginine-glycine-aspartate-4C (RGD-4C) motif into the HI-loop of the fibre-knob, which enhances binding to $\alpha v\beta 3$- and $\alpha v\beta 5$-integrins and enables CAR-independent uptake [34]. RGD-modified viruses have improved oncolytic actions compared to unmodified virus in CAR-negative cancer cells. This strategy has proven to be efficacious for targeting of several solid cancers [35].

2.2.2. Chimeric viruses

The strategy of generating chimeric mutants has the advantage of employing multiple binding motifs from various parental viruses resulting in a broader transduction range of host cells [36]. For example, ovarian cancer cells were more efficiently targeted by adenovirus type 3 (Ad3) that binds to receptors including CD46 and CD80/CD86, but not CAR, and the Ad3 fibre was subsequently inserted in Ad5 to replace the native Ad5 fibre [36, 37]. The resulting chimeric Ad5/3 was more efficient in targeting ovarian cancer cells than Ad5 [38].

2.2.3. Antibody fusion constructs

A novel approach to improve adenovirus selectivity is the use of antibody fusion constructs, where two antibodies are used; one targets adenovirus capsid proteins, for example, using an antifibre knob antibody, and the other targets specific membrane receptors on the tumour cells [32, 33]. One promising cellular target antigen is the folate receptor, which is overexpressed in breast, ovarian, lung, and brain cancer cells [39]. This strategy resulted in improved

selectivity and higher affinity of adenovirus to the tumour cells. A disadvantage of this technique is that the antibody binding to virus is lost in the progeny virions.

2.2.4. Complementation deletions

The most common strategy to generate replication-selective oncolytic adenoviral mutants is by introducing mutations in viral genes that are vital for replication in normal cells but are complemented by the altered cell cycle regulation in tumour cells (**Figure 1**). The first oncolytic adenovirus that was generated (*dl*1520, Onyx-015) was constructed by deleting the viral E1B55K protein, which binds to cellular p53 [15]. Inactivation of p53 is vital for adenovirus replication in normal cells to prevent apoptosis as a defence response to viral infection. In most cancers, p53 is nonfunctional through either direct mutations or mutations of p53-regulatory proteins [40]. It was demonstrated that adenoviral mutants that do not express E1B55K replicate exclusively in cancer cells lacking functional p53. Several versions of E1B55K-deleted mutants have shown promising oncolytic activity in spite of attenuated viral replication in numerous solid cancers. It is now known that E1B55K is crucial for the export of viral mRNA from the nucleus, giving a rational explanation for the limited replication of the virus in cancer cells [41]. Ongoing work is aimed at designing mutants with nonattenuating deletions to improve replication and efficacy in cancer cells. An example is the E1ACR2-deleted mutants that replicate selectively in cells with deregulated pRb-p16 pathway [42, 43]. The deletion of the small pRb-binding CR2-region in the E1A gene prevents binding to pRb, and thus these mutants cannot replicate in normal cells. Several versions of oncolytic adenovirus based on the deletion of E1ACR2 (e.g., Ad5/3Δ24hCG) have been designed and are under clinical evaluation for different types of cancers [43].

3. Prostate cancer–specific oncolytic adenoviruses

Oncolytic adenoviruses provide a promising treatment option for PCa due to their unique mode of action that synergises with current treatment modalities. There are two successful approaches that have been explored when targeting PCa. The first approach was to drive viral replication by prostate-specific promoters replacing the native viral promoter; this strategy is feasible because of the frequent overexpression of AR-regulated genes such as the prostate-specific antigen (PSA) and prostate-specific membrane antigen (PSMA) [44, 45]. Numerous viruses have been developed utilising various combinations of androgen-response elements (ARE) present in these genes [46]. The second strategy is the complementation deletions (see Section 2.2.4).

3.1. Prostate-specific antigen (PSA) regulatory elements

Several specific PSA regulatory elements have been explored to control adenovirus replication, including AREs and the PSA enhancer, which are located upstream of the promoter. The mutant CG7060 was constructed to express E1A from the PSA promoter/enhancer and showed selective replication and cell killing in prostate cancer cells [44]. In animal models using the PCa cell line LNCaP (AR-positive, PSA-expressing), tumour xenografts were grown in mice and were injected intratumourally with CG7060. During the first 2 weeks, the tumour volume increased

slightly followed by a rapid decrease in growth. By 6 weeks, 50% of the mice were tumour-free [44]. In a clinical trial including 20 patients with locally recurrent PCa, CG7060 showed an acceptable safety profile and was not associated with significant toxicity (<grade 3) [18]. In addition, promising anticancer activity was suggested based on the reduction in PSA levels. A more recent and improved version of the CG7060 virus is CG7870, which has the PSA enhancer/promoter controlling E1B expression, while E1A expression is regulated by the rat probasin promoter (AR-regulated) [47]. CG7870 replicates 10^4–10^5 times more efficiently in PSA-positive cells than in PSA-negative cells, which is translated into 10,000 times higher cell killing activity [7, 48]. CG7870 was assessed in phase I and II trials for the management of locally recurrent prostate cancer through intratumoural administration and in hormone refractory metastatic prostate cancer through intravenous administration [19, 49]. In both settings, CG7870 was reported to significantly reduce PSA levels. Moreover, CG7870 synergised with other DNA-damaging therapies including radiotherapy or taxane chemotherapy in preclinical models [5, 7, 48].

3.2. Prostate-specific membrane antigen (PSMA) regulatory elements

PSMA is expressed in the prostate epithelial cell membrane and is significantly elevated in PCa cells compared to normal prostate cells and parallels the increases in Gleason score [50]. There are two identified transcriptional regulatory elements: the PSMA enhancer core (PSME) in the third intron of the PSMA gene (FOLH1) and the 1.2 kb upstream promoter of FOLH1 [51]. PSME is the key element for the prostate-specific expression of PSMA and is negatively regulated by androgens, which explains the high level of PSMA in prostate cancer after castration, unlike the PSA enhancers/promoters, which depend on androgen for activity [52]. The feasibility of using PSME as a regulatory element to control viral replication in PCa tissue has been evaluated by constructing the mutant Ad5-PSME-E1A with replication regulated by PSME-driven expression of E1A [52]. Castrated mice with prostate tumour xenografts received an intratumoural injection of Ad5-PSME-E1A or control virus, resulting in significant tumour regression only in Ad5-PSME-E1A-treated animals [52]. These outcomes suggest that PSME-mediated oncolytic adenovirus may be a promising strategy for management of PCa patients after hormonal therapy failure.

3.3. Prostate-specific chimeric regulatory elements

To further improve on the selectivity of viral mutants, chimeric prostate-specific enhancer-promoter elements were generated and explored [44, 53]. The combination of regulatory elements from PSA and PSMA was named prostate-specific enhancing sequence (PSES) and was inserted in Ad5 to generate Ad-UI1 and Ad-UI2 with the E1A and E4 genes controlled by the PSES [53]. Ad-UI1 is armed with the prodrug-converting enzyme thymidine kinase (TK) from HSV. Ad-UI1 showed selective cytotoxicity against androgen-independent PSA/PSMA-expressing prostate cancer cells in preclinical models of PCa [53].

In another approach, a triplet of prostate-specific enhancers was constructed to regulate adenovirus replication generating Ad[I/PPT-E1A] with E1A under the control of a complex chimeric promoter/enhancer sequence designated PPT [54, 55]. PPT is comprised of the T-cell receptor γ-alternate reading frame protein promoter (TARP) and the PSMA and PSA enhancers.

The chimeric sequence is shielded from interfering adenoviral promoter-sequences by the mouse H19 insulator. Ad[I/PPT-E1A] demonstrated high and prostate-specific replication both in the presence and absence of androgens with promising oncolytic effects in PCa cell lines. Moreover, LNCaP xenograft tumours in mice regressed after intratumoural administration of Ad[I/PPT-E1A].

3.4. Targeted replication of adenovirus through complementation deletions

Several modified versions of Onyx-015 have been designed to develop prostate-specific onco-lytic adenoviruses [15, 56]. For example, Ad5-CD/TKrep is armed with the cytosine deaminase (CD) and TK suicide genes [57]. In a phase I study, the intratumoural administration of Ad5-CD/TKrep was evaluated in locally recurrent prostate cancer in combination with the prodrugs 5-fluorocytosine (5-FC) and ganciclovir [20]. Ad5-CD/TKrep reduced PSA levels with a good safety profile; 44% of patients showed more than 25% decreases in PSA and 19% showed more than 50% decreases in PSA. Tumour cell killing at the administration sites was demonstrated by biopsies 2 weeks later. Interestingly, two patients were cancer free at 1 year follow-up.

Later, a second-generation of Ad5-CD/TKrep was developed; Ad5-yCD/mutTKSR39rep-ADP expressing the adenovirus death protein (ADP) and an improved yeast CD/TK chimeric suicide construct [58]. Ad5-yCD/mutTKSR39rep-ADP showed higher cancer cell killing activity in pre-clinical studies compared to the parental virus. Moreover, in a phase II trial, promising synergis-tic anticancer activity was seen in combination with radiation therapy [21]. Another version of this mutant is (Ad5-yCD/mutTKSR39rep-hNIS), which in addition to the chimeric suicide gene expresses the human sodium iodide symporter (hNIS), which serves as a reporter gene to enable localisation through noninvasive single-photon emission computed tomography (SPECT/CT) [59]. The most recent version of these mutants is Ad5-yCD/mutTKSR39rep-hIL12, which instead of hNIS expresses the human interleukin 12 (IL-12) [60]. IL-12 is a proinflammatory cytokine released by antigen-presenting cells to activate the innate and adaptive immune responses. IL-12 has reported antitumour activity by overcoming the immune suppressive nature of the tumour microenvironment and inhibiting angiogenesis. Local administration of Ad5-yCD/mutTKSR-39rep-hIL12 may evade systemic toxicity of IL-12 while maintaining its therapeutic activity locally. Systemic administration of Ad5/3Δ24hCG, targeting the Ad3 receptor expresses the β-chain of human chorionic gonadotropin (hCGβ), was reported to have anticancer activity in mice with castration-resistant lung metastasis of PCa, resulting in significant survival advantages [43].

4. Challenges using oncolytic adenoviruses for prostate cancer

The promising results from clinical trials with oncolytic adenoviral mutants are, in the majority of cases, derived from localised PCa and intratumoural administration [18, 20, 21]. However, the poor survival outcomes for late-stage PCa patients are due to metastatic lesions in skel-eton and lymph nodes. While the oncolytic mutants readily spread within the tumour tissue after local administration, metastatic lesions need to be targeted through systemic delivery, which is currently not feasible due to the high-affinity binding to erythrocytes, other factors present in the blood, and through elimination of virus from the circulation by the liver [8, 61].

4.1. Preexisting antibodies

A major hurdle in achieving efficient tumour uptake after systemic delivery of oncolytic adenoviruses is the preexisting immunity to virus since the majority of the population has previously been infected with adenovirus. One strategy to overcome preexisitng immunity is to encapsulate the virus in liposomes. It was demonstrated that despite the presence of adenovirus antibodies, liposome-coated virus infected tumour cells in preclinical *in vivo* models [62]. Another strategy, which has also been explored in noncancer research, is the administration of anti-CD20 antibodies to inhibit T cells and deplete B cells from the host. This strategy resulted in enhanced replication of adenoviruses regardless of preexisting adenoviral immunity [63]. A more sophisticated approach is the "Trojan Horse", in which the virus is delivered within a host cell that targets tumours. A similar approach is incorporation of the E1A gene into cytotoxic T lymphocytes (CTL) with expression controlled by the cell activation-dependent CD40 ligand promoter [64]. After transduction of CTLs with E1-deficient adenoviral vectors and activation by CD40, E1A was expressed and infectious virus was produced. Viral replication was tightly associated with CTL activation by its specific tumour-associated antigen, resulting in targeted delivery of oncolytic virus to the tumour [64].

4.2. Binding to erythrocytes

Human erythrocytes express CAR and complement receptor-1 (CR1) that bind to adenovirus with high affinity [65]. The binding significantly decreases the levels of free circulating virus, in turn attenuating viral infection of tumour target tissue. Therefore, erythrocyte binding is a great challenge for systemic administration of oncolytic adenoviruses. To overcome these obstacles, it might be possible to shield the virus with a layer of hydrophilic polyethylene glycol, modifications of the capsid proteins, or as described above with liposome encapsulation [62, 66].

4.3. Uptake by nontargeted healthy tissue

Most adenoviruses are eliminated from the circulation by Kupffer cells through nonreceptor-mediated uptake [67, 68]. For Ad5, up to 90% is taken up by hepatocytes and Kupffer cells in the liver within minutes of intravenous delivery in humans, drastically preventing sufficient amount of virus to reach the targeted tumours [68]. To increase the amount of circulating virus, several strategies have been investigated. One preclinical study explored preadministration of warfarin, which depleted Kupffer cells and prevented hepatocyte binding and consequently improved the anticancer activity of an intravenously administered oncolytic adenovirus [69]. Although warfarin administration may not be feasible in patients, the study demonstrated that circulating levels of Ad5 mutants could be increased by blocking liver uptake. The major key factors associated with liver sequestration of oncolytic Ad5 mutants are the blood coagulation factors IX (FIX) and X (FX) that bind to the capsid proteins and mediate erythrocyte and hepatocyte binding [70]. To avoid these interactions, various chimeric capsid mutants have been generated with altered hexon and/or fibre proteins including the Ad3/Ad11 mutant ColoAd1 (enadenotucirev; PsiOxus) that is currently in phase I–II trials with reported promising outcomes in several solid cancers after systemic delivery [71]. Another mutant Ad5/48 with hexon proteins from Ad48, which have low affinity to FX, demonstrated decreased liver uptake in preclinical models [72].

4.4. Endogenous cytokines

Systemic virus administration stimulates the release of a range of cytokines such as interferons (IFN types 1, 2, and 3) [73]. Their major roles are to induce apoptosis of virus-infected cells and promote resistance to infection in noninfected cells. Moreover, IFNs stimulate the adaptive immune system, mainly the dendritic cells, to initiate long-term immunity. One strategy to overcome the IFN-response is to pretreat the patients with histone deacetylase inhibitors (HDACi) that induce epigenetic changes preventing antiviral cytokine activity at the tumour sites and significantly enhancing systemic efficacy of oncolytic mutants [74]. Delivery of the virus within mesenchymal stem cells derived from the patient may also aid in avoiding the IFN responses since mesenchymal stem cells suppress activated T cells [75].

5. Future directions

The efficacy of numerous oncolytic viruses in cancer management has been established, although only two mutants have been granted market approval to date [15, 17]. Major clinical drawbacks associated with oncolytic adenoviruses are the significant losses of virus after systemic administration resulting in low doses reaching the tumour lesions. In addition, the complexity of designing potent and selective oncolytic viruses without toxicity to normal cells but potent cancer killing activity requires further optimisations. Ongoing work is focused on all aspects of delivering optimised mutants to metastatic lesions. One novel approach is to employ less common serotypes, including Ad3, Ad11, and Ad48, that are more resistant to elimination after intravenous administration. Natural infection with these serotypes is less frequent and preexisting immunity is rare. In addition, the utilisation of other uptake receptors than those of Ad5, CAR, and $\alpha v \beta 3$- and $\alpha v \beta 5$-integrins is an advantage both for improved cancer-cell uptake and decreased erythrocyte and blood-factor binding. A similar approach is the use of chimeric adenoviral mutants including replication-selective alterations of, for example, the Ad5 genome and exchange of capsid proteins from other serotypes such as Ad3 and Ad11. A method for generating cancer cell–selective optimised novel chimeric mutants is "directed evolution" [75]. This concept involves pooling of several serotypes of adenovirus followed by numerous passaging of virus on the cancer cell type of interest, which promotes recombination between serotypes. This process represents an accelerated simulation of the natural selection of viruses, and the most potent mutant can be selected from the resultant viral pools for further study. The methodology can be applied to most epithelial cancer cell lines. To date, a potent oncolytic adenovirus has been generated using this approach, ColoAd1 (enadenotucirev; PsiOxus), which entered phase I–II trials with reported promising outcomes in several solid cancers after systemic delivery [71]. Potency and selectivity on colon cancer cells were significantly higher compared to Onyx-015 [76]. ColoAd1 was selected on colon cancer cell lines and was not evaluated in PCa patients; however, a similar approach using prostate cancer cell lines may lead to the generation of prostate-selective chimeric adenoviruses suitable for systemic administration.

A major advantage of using adenoviruses as anticancer therapeutics is the safety with only self-limiting flu-like side effects [77]. While administration of current oncolytic adenoviral mutants as single agents has not resulted in significant increases in survival, in combination

with cytotoxic drugs and immune factors, efficacy was greatly improved [7, 48]. One strategy to overcome the high level of resistance to anticancer immune responses is to include transgenes into the viral genome, such as GM-CSF and IL-12, to further improve the anticancer activity by boosting antitumour immunity [8, 78]. Arming oncolytic viruses with immune stimulatory factors show promise since intralesional administration of virus might induce a synergistic action between viral oncolysis and antitumour immune responses. This concept is particularly significant for prostate cancer management, as prostate cancer usually does not respond to management with immunotherapeutic agents such as check point inhibitors, due to the immunosuppressive character of this cancer [79]. In addition, the immune responses resulting from cancer cell lysis and death are anticipated to target metastatic tumours even after clearance of the oncolytic virus from the body.

Other issues are the variable susceptibility of tumours to oncolytic adenoviral mutants, likely caused by the specific gene alterations in each tumour type [80]. It may be possible to characterise each patient tumour and select from a panel of oncolytic adenoviruses specifically targeting the identified mutations. A more practical approach to enhance oncolytic efficacy is through combining the mutants with other treatment modalities including cytotoxic drugs and small molecule–targeted therapies [80, 81]. A recent example is the combination of the H101 mutant with a small interfering RNA targeting Bcl2 (siBcl2) [80]. In preclinical studies, the combination resulted in significantly increased tumour cell cytotoxicity and apoptosis compared to either agent alone. *In vivo* tumour xenograft studies demonstrated that combining H101 with siBcl2 significantly reduced tumour growth and prolonged survival.

6. Conclusions

For patients with early-stage prostate cancer, the current treatment modalities are efficient, with 5-year progression-free survival rates of more than 90%. On the other hand, for patients with advanced PCa (stages III and IV), there is currently no effective therapy. Although the latest therapeutic developments for late-stage metastatic PCa have provided a variety of management options that offer significant clinical benefits for patients, the disease still has almost 100% mortality rate at this stage. The median survival after development of hormone resistance is 14 months. Current treatment options have modest effects on survival, extending life by around 2.5–5 months, and are associated with increased treatment costs [82]. Therefore, the need for novel therapies is pressing. Oncolytic viruses have proven potential for the future management of PCa. Several factors make adenoviruses valuable anticancer agents, such as the biology of the viruses is well understood, the viral genome is small and easy to manipulate, and the viruses can induce direct cell death, synergise with apoptosis-inducing chemotherapeutic drugs and stimulate the immune system to develop cancer-specific immune responses. The anticancer mechanisms of adenoviruses are unique without the development of cross-resistance to current therapeutics and have only mild side effects.

Adenoviruses are the most attractive and promising oncolytic virus species that have yet been developed for treatment of different types of solid cancers including PCa. Reports from

phase I–II clinical trials, including PCa patients, demonstrate that these viruses have excellent safety profiles that have been reproduced in thousands of patients. The reported efficacy is promising because of the synergistic interactions between oncolytic adenoviruses and chemotherapy/radiotherapy. Phase III clinical trials are ongoing to assess the efficacy of oncolytic mutants in locally recurrent and high-risk local prostate cancers [21]. If the results of these trials confirm the efficacy and safety, the first oncolytic virus therapy for PCa patients may become a reality in the future. Additionally, arming the viruses with cytotoxic transgenes and immune stimulatory factors represents a promising approach to enhance efficacy in both localised and metastatic PCa. A recent advancement in the development of optimised oncolytic viruses is the generation of chimeric viruses by utilising serotypes that are more resistant in the circulation. An effective but labour-intense approach is to generate chimeric oncolytic adenovirus with enhanced potency, circulating half-life and selectivity to specific cancer types by directed evolution [76]. The major drawback of oncolytic adenoviruses is the disappointing anticancer activity against distant metastatic tumours after systemic administration, and by employing novel chimeric serotypes, it may be possible to develop superior mutants with properties suitable for intravenous delivery. The adenoviral mutants Ad5/3Δ24hCG demonstrated promising anticancer activity in preclinical metastatic hormone-resistant PCa models, which prolonged survival *in vivo* [43]. If the same results are reproduced in patients, a great impact on the management of metastatic PCa can be anticipated. We predict that in the near future oncolytic adenoviruses will be a treatment choice for this indication and will add to the novel therapies that aim to cure late-stage castration-resistant prostate cancer.

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