Review

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Adenovirus vector system: construction, history and therapeutic applications

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ABSTRACT

Since the isolation of adenovirus (AdV) in 1953, AdVs have been used as vectors for various therapeutic purposes, such as gene therapy in cancers and other malignancies, vaccine development and delivery of CRISPR-Cas9 machinery. Over the years, several AdV vector modifications have been introduced, including fiber switching, incorporation of ligands in the viral capsid and hexon modification of the fiber, to improve the efficiency of AdV as a vector. CRISPR-Cas9 has recently been used for these modifications and is also used in other adeno-associated viruses. These modifications further allow the production of AdV libraries that display random peptides for the production of cancer-targeting AdV vectors. This review focuses on the common methods of AdV construction, changes in AdV tropism for the improvement of therapeutic efficiency and the role of AdV vectors in gene therapy, vaccine development and CRISPR-Cas9 delivery.

TWEETABLE ABSTRACT

Adenovirus vectors have been used for vaccine development, gene therapy and delivery of the CRISPR-Cas9 system. Several modifications have been introduced to enhance the efficacy of AdV vectors for genome editing and cancer therapy, including fiber switching, incorporation of ligands in the viral capsid and hexon modification of the fiber.

KEYWORDS:

adenovirus vectors ● Ad5 ● AdV ● AdV modification ● AdV tropism ● Cre-lox method ● CRISPR-Cas9 ● gene therapy ● homologous recombination ● vaccine development

A vector is a 'particle' (typically a plasmid, cosmid or virus) that is employed as an artificial means of delivering a specific DNA segment into a host cell. Many natural and artificial vectors have been used for therapeutic purposes and vaccine preparation, but viral vectors are the most efficient gene delivery systems because of their specificity in targeting particular cell types and tissues. Viral vectors may be manipulated to carry genes whose expression is inevitable in therapeutics [1]. Several viral vectors have been investigated thus far, including adenoviruses (AdVs), lentiviruses, retroviruses, herpes simplex virus, adeno-associated virus and poxviruses. Retroviral vectors are used for integration of genetic material in infected cells; however, for transduction of these retroviral cells, mitotic division needs to be in progress, which is a significant limitation of these vectors. Similarly, lentiviral vectors such as HIV demonstrate unpredictable genome integration in the host genome. By comparison, AdV has the ability to transport cargo to dividing and nondividing cells both *in vitro* and *in vivo*. The main limiting factor is 'precipitating immune elimination of cells' when it is used *in vivo*. Adeno-associated viral vectors also deliver cargo to both dividing and nondividing cells with very limited immune response but are restricted by cargo capacity. The choice of viral vector in clinical use depends on its ability to express foreign genes and transgenes as well as its safety profile and stability under severe conditions. Among the viral vectors, AdV vectors are the most frequently used [2].

AdVs were first detected in human adenoid tissue and were manipulated to use as gene delivery vehicles in the 1980s [3]. The Adenoviridae family constitutes five genera: *Aviadenovirus* (infecting birds), *Mastadenovirus* (infecting mammalian species), *Atadenovirus* and *Siadenovirus* (both introduced in 2002) and *Ichtadenovirus*. AdVs that infect humans are classified into six (A–F) species, which are further divided into 50 serotypes [4]. Two human AdV serotypes – namely, AdV serotype 2 (Ad2) and AdV serotype 5 (Ad5) – from species C are most commonly used to make recombinant AdV vectors. These vectors usually cause mild diseases of the respiratory system and no oncogenesis. This makes AdVs the best cargo delivery system, and the mild immune response to AdVs makes them best suited for use in *in vivo* gene therapies [5].

Gene therapy is frequently used in cancer treatment. Advancements in gene therapy have utilized two strategies, the first of which is the replication-defective vector strategy, which is used to express a therapeutic transgene in a replication-defective viral vector. Tumor cells are transduced with the transgene, and its expression produces anticancer affects. AdVs are widely used as replication-defective

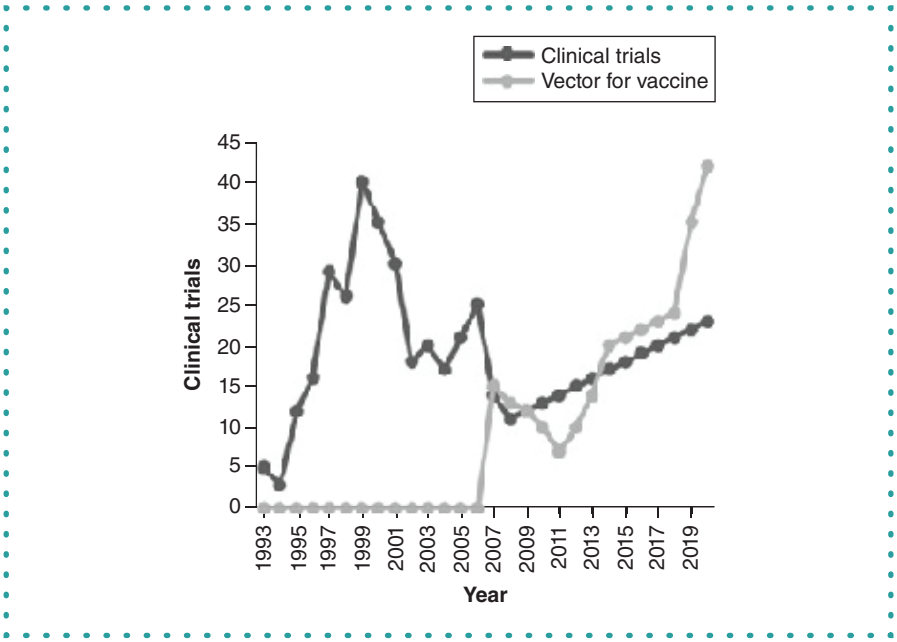

Figure 1. Trends in the rise and fall of AdV vectors in clinical trials of gene therapy and vaccine delivery for various diseases from 1993 to 2019. Data taken from [12,13].

vectors, with the main benefit of high expression of the therapeutic gene inside the tumor cell and lower vector protein expression, making AdVs safer vectors [6]. The safety profile of AdV vectors is further increased by knocking out the *E1* gene, which results in replication-defective AdV vectors. Deleting the *E1* gene also allows AdV vectors to accommodate 7.5 kb of foreign DNA, and this length can be further increased through more deletions. Another advantage is that these AdV vectors can be filtered to a very high load of 10^{13} particles/ml, and these viruses infect both mitotic and nondividing cells. The second strategy is the replication-competent strategy in which lytic viruses are engineered to generate oncolytic therapeutic vectors that replicate specifically in tumor cells and induce lysis. The main benefit is that this amplifies the therapeutic gene inside the tumor cell and helps to spread transgenes in the whole tumor [7].

The characteristics of Ad2 and Ad5 as vectors have led to substantial interest in their use as gene delivery and vaccine development systems. Several techniques have been developed to favorably manipulate the AdV genome; however, there are still some limitations to the use of AdVs as vectors. Species C AdV vectors possess native tropism for coxsackievirus and AdV receptor and integrin $\alpha v\beta$, which prohibits cell-specific targeting of AdV vectors. Furthermore, some target cells express very low levels of cell surface receptors that cause weak infection and are attacked by innate and acquired immune responses in *in vivo* gene therapy. Several AdV modifications have been performed to address these disadvantages and unleash the full potential of AdV vectors [1]. This review focuses on the construction and genome modification of AdV vectors and their application in gene therapy, vaccine development and CRISPR-Cas9 delivery.

History of AdV as a vector

Since the isolation of AdV in 1953, AdVs have emerged as a promising gene delivery vehicle. Of the wide variety of human and nonhuman AdVs, Ad5 is widely used and is the most characterized, with well-known biology [8]. The AdV genome has been progressively modified from its wild-type over the last three decades to improve its safety and therapeutic profile. A decrease in immunological response is seen by progressively removing nonessential DNA regions, from the replication region (first generation) to the gene (third generation). As a result, AdV gene insertion capacity has been enhanced from approximately 7 to 36 kb [9].

Between 1993 and 1998, clinical trials for AdV vectors in gene therapy continued to increase. However, in 1999, a sudden dramatic decrease occurred because of the death of Jesse Gelsinger (Figure 1) during ongoing therapy for treatment of ornithine transcarbamylase deficiency [10]. At that time, safety concerns regarding AdV vectors were re-evaluated by the NIH, which conducted AdV protocols to design safer and more effective AdV vectors. The therapeutic target then moved from monogenic to marketable transient therapies, at which time AdVs became interesting candidates for vaccine production and therapy for cancer, arthritis and vascular dysfunction. In 2007, AdV vectors were majorly (80%) being used in clinical applications for cancer therapy [11].

In the 1980s, scientists began to generate Ad5 vectors by stripping their replicative genes and inserting them into cell lines; hence, these viruses can replicate in a specific cell line. This also made room for transforming genes of interest. The death of Jesse Gelsinger during gene therapy was the result of inoculation with 38 trillion viral particles, which resulted in inflammation of his entire body due to a hyperactive immune system. This led to the idea of using AdV vectors in vaccine development. In vaccine production, the main advantage

is that no additional adjuvants are required. The AdV vector itself drives inflammation, which is controlled by giving low doses. Owing to these benefits, in the early 2000s, some vaccine developers began to use AdVs as a vehicle for vaccine delivery. In 2007, the first HIV vaccine was developed using AdV vector but the desired results were not obtained [14]. This put a stop to the use of AdVs as tools for vaccine development for the next 5 years. In 2014, the Chinese company CanSino Biologics generated an AdV-based vaccine for an Ebola outbreak, but the vaccine was approved only for emergency use [15]. These results encouraged scientists to use AdVs as vehicles for developing COVID-19 vaccines in the recent outbreak.

Techniques for generating AdV vectors

The construction of recombinant AdV vectors remains a complicated and tedious process. Over the years, several strategies have been developed for their efficient production and utilization. Some of the approaches used for AdV vector production as well as their possible advantages and limitations are discussed in the following sections.

In vitro ligation method

In vitro ligation is a classical method for directly inserting the gene of interest into the AdV genome [16]. It is commonly used to ligate large linear fragments of AdV DNA to the plasmid that occupies the gene of interest and the remaining part of the AdV genome. The first step is plasmid construction, which is performed using the left region of the AdV genome (particularly left inverted terminal repeats), the E1A enhancer sequence and a packaging signal. After insertion of the gene of interest into the plasmid, both the AdV genome and the plasmid are treated with the restriction enzyme ClaI, whose restriction sites are within the *E1* region of AdV. This treatment results in the formation of fragments, which are allowed to ligate *in vitro*. Hence, a portion of the AdV E1A region gets replaced with a foreign gene, and the modified AdV genome is then transfected into human embryonic kidney (HEK) 293 cells [17].

Improved *in vitro* ligation methods using two plasmids have also been developed. In these methods, the vector plasmid contains the complete AdV genome, and restriction sites for I-CeuI, SwaI and PI-SceI are introduced along the *E1* deletion site of the plasmid. The second plasmid used is a shuttle plasmid, which contains the gene of interest inserted between multiple cloning sites of I-CeuI and PI-SceI. Treatment with these restriction enzymes produces fragments that ligate, and the inserted gene gets replaced with the *E1* region of the vector plasmid, producing a recombinant vector [18].

The *in vitro* ligation method is frequently used to produce first-generation AdV vectors. This approach is simple and can be easily scaled up, but inefficient ligation and scarcity of unique restriction sites limit its use [19].

Homologous recombination method

Homologous recombination involves recombination between two DNA molecules in permissive cell lines. The shuttle vector carrying the gene of interest and left end of the AdV genome undergoes recombination with linear, purified AdV DNA with the right end of the AdV genome in HEK 293 cells to produce the desired viral vector (Figure 2) [20]. An improvement to this technique is the use of two plasmids, which undergo homologous recombination in bacterial cells, particularly *Escherichia coli*. The gene to be inserted is cloned in the shuttle vector with the left region of the AdV genome. The recombination takes place between the shuttle plasmid and genomic plasmid carrying the entire AdV genome with appropriate deletions [21]. Another advance strategy involves homologous recombination between the AdV genome and yeast artificial chromosome [22]. The homologous recombination method is effective and widely used, but low efficiency of the recombination event and time-consuming plaque purification are major barriers to its use.

Cre-lox recombination method

Cre-lox recombination uses the P1 bacteriophage to accomplish the desired recombination *in vitro* [23]. This system has two main components: loxP sites, which are the sites where crossing over takes place, and Cre recombinase, a protein of P1 phage that facilitates recombination between two loxP sites. Recombinant AdV with two loxP sites and a shuttle vector containing the left region of AdV, gene of interest and a loxP site are co-transfected into helper cell lines such as HEK 293 cells, which express Cre recombinase of the P1 phage (Figure 3). Two recombination events take place, the first of which is intramolecular and occurs within AdV DNA between loxP sites. This eventually leads to the second recombination, which usually involves the loxP site of the shuttle vector and that of the first product and results in production of the required AdV with the gene of interest attached [24]. Cre-loxP recombination is a powerful tool for producing highly efficient recombinant AdV vectors in a relatively short time, but high persistence of the parent virus is a major drawback associated with this method [25].

Bacterial artificial chromosome technology

AdV genome manipulation using bacterial artificial chromosome (BAC) technology has proven to be a successful alternative to conventional genetic engineering of recombinant AdVs. BAC technology enables the construction of recombinant genomes using a small amount of AdV DNA isolated from early passage isolates. This allows quick access to molecular clones of virtually any AdV strain or variant, including isolates with a high risk of changes brought on by adaptation to tissue culture or previously mutated [26]. Since BAC technology seems to be very helpful for quickly and accurately manipulating AdV genomes, the majority of applications have been adapted for Ad5 [27].

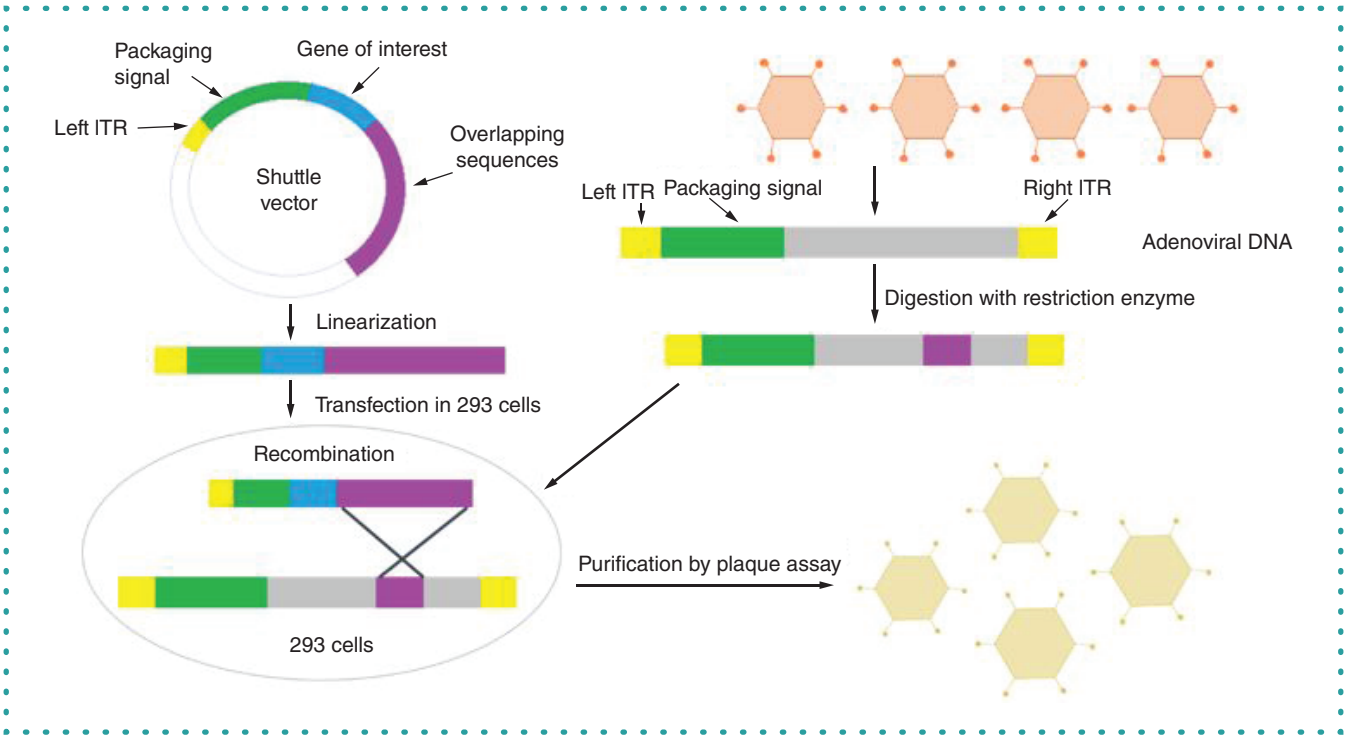

Figure 2. Construction of E1-substituted AdV vector by homologous recombination method in human embryonic kidney 293 helper cells. The desired gene is first cloned in a shuttle vector containing a 5′ inverted terminal repeat, a packaging signal and a region for homologous recombination. After linearization with a unique restriction enzyme, the shuttle vector is co-transfected with AdV gDNA and digested with a restriction enzyme to release the *E1* region from the DNA backbone. Homologous recombination in human embryonic kidney 293 cells results in the generation of recombinant clones containing entire sequences of AdV except the *E1* region, which is replaced by the transgene.

To generate the BAC AdV genome, a cassette with positive selection markers for kanamycin resistance and galactokinase is first amplified by PCR, creating flanking homologous regions, and then introduced into the BAC by homologous recombination and positive selection for kanamycin resistance. Following PCR amplification, the sequence containing the modification flanked by homologous regions replaces the galactokinase–kanamycin resistance cassette through homologous recombination. Isolation of the BAC containing the modified AdV genome is made possible by negative selection against galactokinase [28].

Changes in AdV tropism

AdV vectors, and specifically Ad5 (because of its known genetic background), are being used in gene therapy. However, AdV vectors have several limitations, including low efficiency with regard to genetic modification of T cells and liver tropism, which results in an enhanced innate immune response against the vectors, severe liver damage and increased systemic toxicity. These limitations hamper the safety and efficacy of AdV vectors *in vivo* and are dealt with using fiber gene modification of AdVs to target specific cells, including neurons and hematopoietic stem cells [29], and hexon modification to decrease hepatic toxicity. The modifications used involve gene deletions, gene replacements or specific mutations.

Fiber switching of Ad5 is done by replacing part of the Ad5 capsid with part of the non-Ad5 capsid. Pseudotyping of the Ad5 fiber with that of Ad3 results in coxsackievirus and AdV receptor-independent infectivity, leading to enhanced gene transfer in a wide range of cancer cell types [30]. Moreover, chimeric Ad5 vectors are being constructed with fibers of Ad35 that use CD46 receptors to infect the majority of cancer cells [31]. Similarly, chimeric Ad3/Ad11p vectors demonstrate enhanced selectivity for colon cancer cells [32]. The replaced AdV serotypes in the chimera determine the tissue tropism of the chimeric virus. Other cellular attachment receptors for tissue tropism of various AdV strains include CD80, CD86 [33] and CD46 [34], sialic acid and p50 [35].

Another modification for retargeting AdV vectors involves incorporation of ligands in the viral capsid. These targeting ligands are specifically incorporated into minor capsid protein IX as well as the HI loop and C-terminus of the fiber protein, Arg-Gly-Asp loop (also known as RGD loop) of the pentose base and L1 loop of hexon. Incorporating targeting ligands in the Arg-Gly-Asp loop of AdV is the most effective modification for increasing AdV vector infectivity (Figure 4). However, the specific cancer-targeting ligands for AdV vectors are mostly unknown. Therefore, AdV libraries displaying random peptides have been developed to produce cancer-targeting AdV vectors. The AB loop of the fiber protein is also modified to inhibit the binding of AdV vectors to natural receptors, thereby decreasing the native tissue tropism of AdV [36]. To decrease liver infection by AdV vectors, specific mutations are inserted in the coagulation factor X-binding

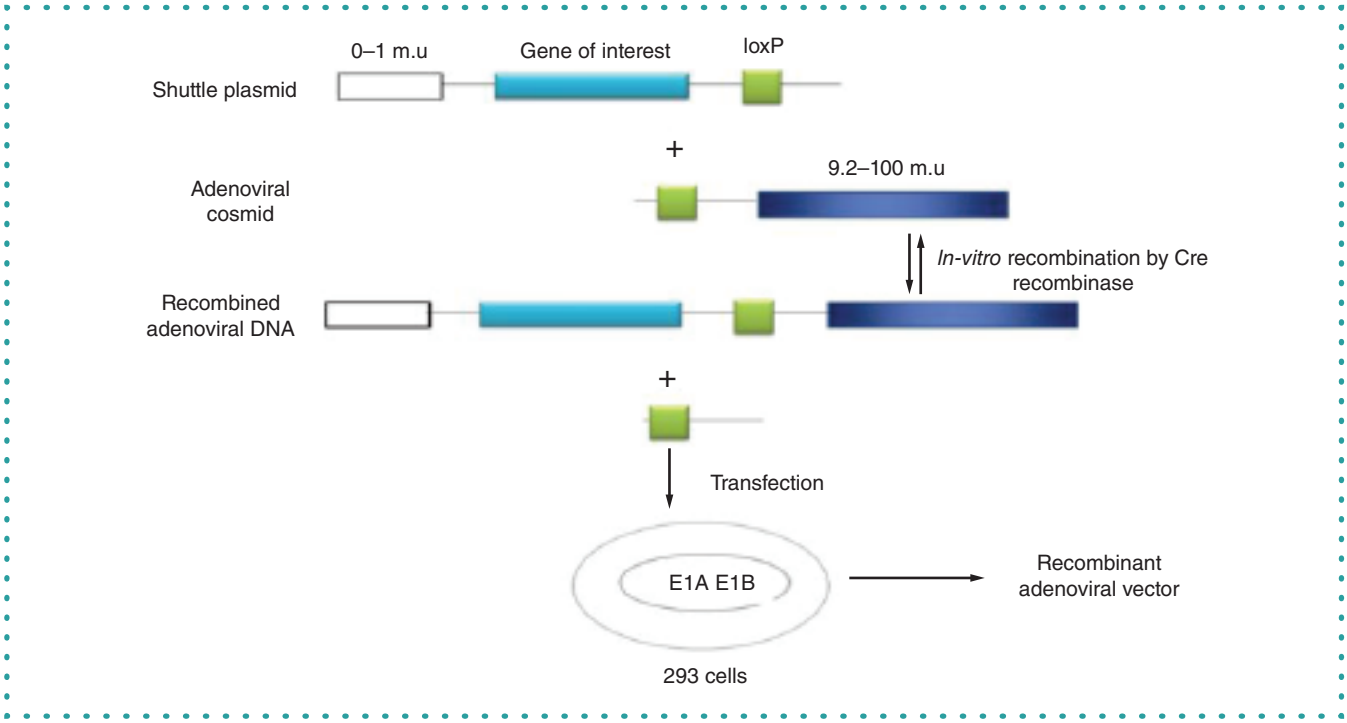

Figure 3. AdV construction by Cre-loxP recombination. The shuttle plasmid includes the AdV 5′ inverted terminal repeat and a packaging signal, gene of interest and a single loxP sequence. The AdV cosmid has a single loxP sequence as well as 9.2–100 mu of the AdV genome with a deletion in the E3 region. The gene of interest is first cloned into a shuttle plasmid, which is then linearized by a restriction enzyme, such as NheI, and recombined *in vitro* with ClaI-digested AdV cosmid. Cre recombinase can generate the full-length recombinant AdV vector *in vitro* by exchanging the linearized sections distal to the loxP site in these two molecules. The recombined DNA is transfected into human embryonic kidney 293 cells for packaging, and homogeneous recombinant AdV can be produced in 7–9 days without plaque purification.

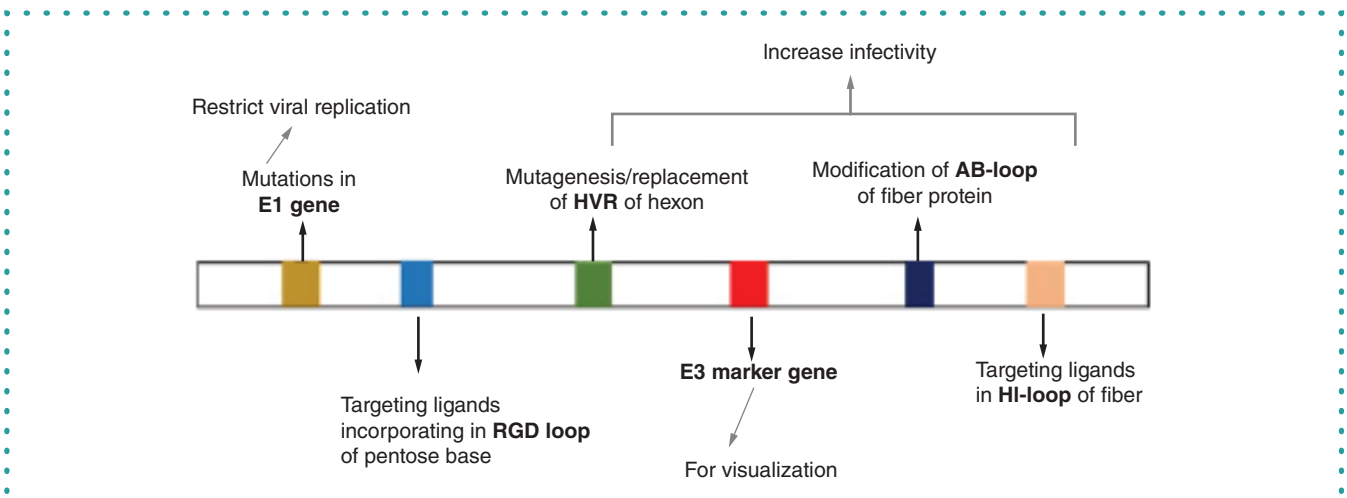

Figure 4. AdV genome with specific modifications for increased efficiency.

domain of the hypervariable regions of AdV hexon or the hypervariable regions of one serotype of AdV are replaced with the hypervariable regions of another serotype [37].

Currently, the most efficient and straightforward method being used for these modifications is the CRISPR-Cas9 genome editing method. The CRISPR-Cas9 system uses only an sgRNA, which guides Cas9 nuclease to the target gene sequence in AdV, where it introduces breaks in dsDNA [38].

Table 1. Oncolytic adenovirus vector therapy in cancer.

Serial no.	Target	Oncolytic AdV vector	Modification	Transgene	Ref.
1	Colon cancer, NSCLC, bladder cancer, renal cancer	Colo-Ad1	Ad11p/Ad3	–	[4]
2	Prostate cancer	HAd5-yCD/mutTKSR39rep-hIL-12	E1B-55K	CD-TK/hIL-12	[36]
3	Pancreatic cancer	LOAd703	5/3-Δ24	CD40L and 4-1BBL (CD137L)	[43]
4	Metastatic melanoma	Ad5/3-D24-TNF-α-IRES-IL2	TILT-123	–	[44]
5	Bladder cancer	CG0070	E2F-E1A	GM-CSF	[45]
6	Advanced neoplasms	ONCOS-102 cyclophosphamide	5/3-Δ24	GM-CSF	[46]
7	Brain tumors	DNX-2401 with IFN-γ	E1A-Δ24 RGD	–	[47]

Various oncolytic AdV vector-based transgene modifications in targeted diseases are given in this table. In the case of oncolytic AdV vector-based cancer therapy, the researchrs constructed AdV vectors with the ability to reproduce favorably that could aid in the destruction of cancer cells via lytic replication in clinical trials.
AdV: Adenovirus; NSCLC: Non-small-cell lung cancer; RGD: Arginine–glycine–aspartic acid.

Table 2. Adenoviral vector therapy in several noncancerous diseases.

Serial no.	Target	AdV vector	Modification	Transgene	Ref.
1	Cystic fibrosis	HAd5-CB-CFTR	E1 deleted	CFTR	[53]
2	Parotid salivary dysfunction	HAd5-hAQP1	E1 deleted	hAQP1	[54]
3	Varicose ulcers	HAd5-PDGF-B	E1 deleted	PDGF-B	[55]
4	Macular degeneration	HAd5-PEDF	E1, E3 and E4 deleted	PEDF	[56]
5	Myocardial infarction, angina pectoris	HAd5-VEGF	E1–E3 deleted	VEGF-D	[57]

AdV vector-based transgene modifications in targeted diseases.For investigating the efficiency of AdV vector-based disease therapy in clinical trials, the authors constructed AdV vectors with the ability to reproduce favorably and to destroy targeted cells via the process of viral lytic replication.
AdV: Adenovirus.

A study demonstrated the use of CRISPR-Cas9 and Gibson assembly to produce gene deletions, replacements and site mutations in Ad5-based vectors [39]. This study also demonstrated the ability of the Gibson assembly to ligate linearized vectors with mismatched base pairing in overlapping ends and DNA fragments. The CRISPR-Cas9 and Gibson assembly system proved to be a similarly useful tool in the *in vivo* homology-directed repair pathway. In another study, the CRISPR-Cas9 system was used to perform *in vitro* modification of the Ad5 fiber genome [40]. Chimeric Ad5F35 AdV was produced by replacing the knob and shaft of the fiber gene of Ad5 with those of Ad35. This method resulted in targeted AdV genome editing and can also be applied to other adeno-associated viral, lentiviral and herpesviral vectors.

Application of AdV vectors

Current AdV vector-based therapeutic challenges

AdV is used as a common vector in gene therapy for the treatment of various cancers (e.g., lung, pancreatic, prostate) and other diseases (e.g., cystic fibrosis, macular degeneration). Ad2 and Ad5 are the most used serotypes for cancer treatment. AdV vector genome remains only as episomal DNA in the host cell nucleus and does not integrate into the host chromosome, so genotoxicity related to AdV vector insertion can be avoided. Moreover, AdV vector genome persists predominantly as replication-defective monomeric genome in host cells [41]. Basically, oncolytic AdV vectors are constructed such that they can reproduce favorably in cancer cells, and through the process of lytic virus replication, cancer cells can be easily destroyed (Table 1). Several trials indicate that replication-competent and replication-defective AdVs have therapeutic action and are safe to use [42].

In cancer, the p53 protein is inactivated by mutation of the *TP53* tumor suppressor gene. Recombinant human AdV *p53* is a human Ad5 in which the *p53* gene is inserted locally at the site of lesions and was the first commercially available recombinant product used in the treatment of liver cancer and epithelial ovarian carcinoma [48]. AdV vector-based gene therapy has also been evaluated in the treatment of prostate cancer via encoding the suicide gene at the cancerous site. This methodology is proven to be more effective than the use of the conventional version of Ad-p53 to kill prostate cancer cell lines and inhibit tumor progression [49].

In 1992, a first-generation AdV vector was used for delivery and expression of α1AT in the hepatocytes of an individual with α1AT deficiency [50]. In another study, an hAd5 vector with an E1–E5 deleted region was used for delivery of an α1AT gene to lung tissues [51]. In the case of cystic fibrosis, AdV vectors have been used to deliver the *CFTR* gene to lung tissues [52]. Several AdV-based clinical trials are ongoing (Table 2).

Since their initial use in therapeutic applications, AdV vectors have faced many challenges. The rising prevalence of pre-existing exclusion of AdV vectors was considered to be a major concern regarding their use in therapeutic applications. Other concerns remain with regard to many AdV-based therapies, such as the potential of vectors to re-gain replication proficiencies, nonspecificity and immunodom-

inance. With a complete understanding of viral immunomodulatory genes and their cellular targets, issues related to the specificity of AdV vectors and the immune system's response against such vectors can be solved [4].

Delivery of CRISPR-Cas9 by AdV vectors

AdV vectors are dsDNA vectors with a large packaging capacity, which makes AdV vectors suitable for integration of large genes as well as gene editing strategies [58]. The AdV genome contains inverted terminal repeat sequences at both 5′ and 3′ ends and has four critical sites: E1, E2, E3 and E4. Therefore, AdV vectors have been used to deliver CRISPR-Cas9 machinery to specific cells for gene therapy. Other viral vectors used for the delivery of this system include lentiviruses and adeno-associated viruses. The CRISPR-Cas9 system is an RNA-guided nuclease system and contains small RNAs (20-nucleotide sequences) known as gRNAs to guide Cas9 endonuclease to specific target DNA sequences [59]. AdV vectors help in the delivery of gRNA and *Streptococcus pyogenes* Cas9 nuclease to the target site [60].

In Ad5, the E1 genomic region is replaced with a Cas9 sequence along with a strong cytomegalovirus promoter and poly-A sequence, whereas the E3 region is replaced with a gRNA sequence along with a U6 promoter to form a vector for delivery of the CRISPR-Cas9 machinery (Ad5-Cas9-gRNA). A second vector contains an EF1α promoter and the desired gene. The desired gene is flanked by sequences that are homologous to the flanking ends of the target gene. For knock-in of the desired gene, the second vector is co-transfected along with the first vector containing the CRISPR machinery [61].

After formation of recombinant plasmids, these plasmids are digested with PacI, and the viral genome is released. AdV particles with a gRNA and Cas9 sequence are formed by transformation of HEK 293 T cells. Target cells are then infected with these viral particles for delivery of the CRISPR machinery [62]. Co-injection of first and second vectors containing viral particles (Ad5Cas9-gRNA and Ad5-EF1α-desired gene) guides Cas9 to the target gene site for generation of a double-strand break and DNA repair via a homology-directed repair mechanism [63].

Vaccine development

The ability of AdVs to influence the innate and adaptive cellular and humoral immune systems has been successfully utilized in vaccine development against viral pathogens. In AdV vector-based vaccines, the virus is rendered replication-defective by deleting the *E1* gene, which impairs the ability of AdV to multiply in the host cells but does not affect the infectivity of the virus. The antigen is then placed with a strong heterologous promoter in an expression cassette that is primarily inserted in the *E1* locus of the AdV genome. BAC-based recombineering, plasmid-based homologous recombination in *E. coli* and *in vitro* Gateway recombination are the most commonly used methods for inserting antigens in AdV vectors using genetic engineering [64]. Human Ad5 vectors were the first AdV vectors to be used in preclinical and clinical vaccine trials; however, the presence of neutralizing antibodies against human Ad5 led to the use of nonhuman AdVs such as chimpanzee AdVs (ChAds), canine AdVs, fowl AdVs, porcine AdVs and ovine AdVs. Of these, ChAds are most commonly used in clinical trials owing to various advantageous properties, including ease of culturing in human cell lines and low seroprevalence rates in humans [4].

ChAds have been used in vaccine development against several infections, including AIDS, Ebola, malaria, hepatitis C, rabies, Zika, Middle East respiratory syndrome coronavirus and severe acute respiratory syndrome coronavirus [59]. Ad26, Ad35 [65], chimeric Ad5H3 and ChAds are being used for the development of vector-based vaccines against AIDS. Chimeric and ChAd-based AIDS vaccines successfully induce antigen-specific cellular and humoral immune responses [66]. To date, several phase I clinical trials are being done to deliver HIV-1 vaccines using ChAds, Ad25 and Ad36; however, no trials are using chimeric AdV vectors.

Currently, several AdV vector-based vaccine candidates are being developed against severe acute respiratory syndrome coronavirus 2. These vaccines provide protective immunity against COVID-19 with minimal side effects and are authorized for emergency use in the COVID-19 pandemic. Several severe acute respiratory syndrome coronavirus 2 vaccines – namely, a human Ad5-based COVID-19 vaccine (Ad5-nCoV) by CanSino Biologics [67], ChAdOx1 nCoV-19 by AstraZeneca [68], Ad26.COV2-S by Janssen Pharmaceuticals [69] and Sputnik V [70] – have been developed using HuAd5, ChAds, Ad26 and recombinant Ad26 and Ad5 vectors, respectively. All of these vaccines are in phase III clinical trials and provide robust humoral and cellular immunity.

Conclusion

Because of their safety profile and expression, AdVs are the most efficient gene delivery vehicles. By providing efficiency in cancer and gene therapy, these vectors have opened new avenues in gene delivery, vaccine development and molecular oncology therapy by targeting the oncolytic genes at molecular level. Oncolytic AdV vectors have announced their importance as the best possible medicinal candidates for enhancing the treatment of cancerous cells. AdVs have also been valuable in vaccine development for emerging infectious viral diseases. Therefore, these vectors are continuously being improved to overcome their limitations including toxicity, pre-existing immunity challenges and construction hurdles.

Future perspective

As the research on AdVs has progressed, our understanding of certain aspects has increased. These vectors can be improved by combining with CRISPR technology for gene therapy by knocking out virulent genes and creating space for insertion of the desired gene.

By doubling the packaging capacity, inefficient production methods can be improved. For this purpose, researchers are using a super-enhancer approach.

To avoid an aggressive immune response, chimeras may become useful in engineering AdV vectors that can selectively evade substantial viral traps (i.e., Kupffer cells). The sequestration of AdV vectors in liver cells, dendritic cells or lymphocytes upon intravenous injection can be avoided by modifying AdV tropism and directing the virus to new receptors. The possibility to prevent AdV vectors sequestration is enhanced if different sensitive targeting ligands are found. Research is ongoing with regards to all these approaches and promises a bright future for AdV vectors.

Executive summary

- The safety profile and expression of adenoviruses (AdVs) make these vectors efficient gene delivery systems.
- Oncolytic AdV vectors effectively remove cancer cells and deliver the genes necessary for removal of tumor cells.
- Modifying AdV vectors using CRISPR technology enhances their efficiency in vaccine development, oncolytic therapy and gene delivery.
- Because of their large packaging capacity, AdVs are used to deliver CRISPR-Cas9 technology.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending or royalties.

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• **Includes information regarding adenovirus (AdV) vectors and how they offer several advantages over other viral vectors, including a broad range of tissue tropism, well-characterized genome and ease of genetic manipulation.**

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