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Adeno-associated virus (AAV) vectors in cancer gene therapy

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ABSTRACT

Gene delivery vectors based on adeno-associated virus (AAV) have been utilized in a large number of gene therapy clinical trials, which have demonstrated their strong safety profile and increasingly their therapeutic efficacy for treating monogenic diseases. For cancer applications, AAV vectors have been harnessed for delivery of an extensive repertoire of transgenes to preclinical models and, more recently, clinical trials involving certain cancers. This review describes the applications of AAV vectors to cancer models and presents developments in vector engineering and payload design aimed at tailoring AAV vectors for transduction and treatment of cancer cells. We also discuss the current status of AAV clinical development in oncology and future directions for AAV in this field.

1. Introduction

Cancer, a large group of diseases characterized by the unregulated proliferation and spread or metastasis of abnormal cells, collectively represents a major worldwide healthcare problem. In the U.S. alone more than 1.5 million cases are diagnosed each year, and cancer overall has a 5-year relative survival rate of 68%, making it the second leading cause of death after heart disease [1]. Standard treatments include surgery, chemotherapy, and radiotherapy; however, these are often incapable of completely eradicating a malignancy [2] and can be accompanied by serious side effects [3]. Thus, there is a strong unmet medical need for the development of novel therapies that offer improved clinical efficiency and longer survival times in patients afflicted with disease.

Gene therapy, defined as the introduction of genetic material into a target cell for therapeutic benefit, is a very promising treatment for many diseases, including monogenic diseases, cancer, cardiovascular disease, and neurodegenerative diseases. To date, more than 2000 clinical trials employing gene transfer have taken place and in general have established that a number of vehicles or vectors are safe [4,5]. Furthermore, the majority (64%, n = 1415 [6]) of gene therapy clinical trials to date have targeted cancer — including lung, skin, neurological, and gastrointestinal tumors — and have utilized a variety of therapeutic strategies such as anti-angiogenic factors, tumor suppressors, immunostimulation, and oncolytic viruses. In 2015, the first recombinant viral therapy — an oncolytic herpes virus for the treatment of melanoma — received regulatory approval in the U.S. [7].

For cancer gene therapies to be increasingly successful, however, a major hurdle must be overcome: the development of gene delivery vectors that can safely, efficiently, and specifically deliver genetic material to the target cells. Non-viral vectors can be easily produced at a large scale and are readily amenable to engineering or enhancement of their functional properties *via* chemical modifications; however, they suffer from a low delivery efficiency and in some cases cell toxicity [8]. On the other hand, viral vectors harness the highly evolved mechanisms that the parental viruses have developed to efficiently recognize and infect cells and offer several advantages, which make them suitable for both therapeutic application and as tools for biological studies; however, their delivery properties can be challenging to engineer and improve. That said, viral vectors have been used in the majority (over 68% [6]) of gene therapy clinical trials, and the most frequently used have been based on adenovirus, retrovirus, vaccinia virus, herpesvirus, and AAV [9].

AAV vectors in particular have been increasingly successful due to their gene delivery efficacy, lack of pathogenicity, and strong safety profile [10]. As a result of these properties, AAV vectors have enabled clinical successes in a number of recent clinical trials that have established

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the promise of gene therapy in general, including for the treatment of diseases such as Leber's congenital amaurosis (LCA) [11,12], where over four phase I and I/II clinical trials have demonstrated safety and long-term (over five years) improvement in retinal and visual function; hemophilia B, targeted in several phase I and phase I/II clinical trials that have shown long-term efficacy and no toxic effects [5,13]; and the Sanfilippo B syndrome, where gene expression and consequently improved cognitive development have been sustained for at least a year and are still ongoing (Pasteur Institute Phase I/II trial, unpublished). Moreover, alipogene tiparvovec (Glybera; uniQure), a gene therapy for lipoprotein lipase deficiency (LPLD) that employs an AAV vector, received regulatory approval by the European Medicines Agency in 2012 [14]. AAV vectors may also offer a strong potential for the treatment of cancer, and as presented in this review, their excellent gene delivery properties have been harnessed for *in vitro* cancer studies, *in vivo* pre-clinical cancer models, and more recently cancer clinical trials under development.

2. Adeno-associated virus (AAV) and AAV vectors

2.1. AAV biology

AAV is a single-stranded DNA parvovirus with a 4.7 kb genome (Fig. 1A) composed of the *rep* and *cap* genes flanked by inverted terminal repeats (ITRs) [15]. The *rep* gene encodes non-structural proteins involved in viral replication, packaging, and genomic integration, whereas the *cap* gene codes for structural proteins (VP1, VP2, VP3) that assemble to form the viral capsid, which serves as the viral gene delivery vehicle. Additionally, an alternative open reading frame nested within the *cap* gene encodes the assembly activating protein (AAP), involved in the targeting and assembly of capsid proteins [16]. Following cellular entry through cell surface receptor-mediated endocytosis, endosomal escape, trafficking to the nucleus, uncoating, and second DNA strand synthesis, the virus can enter its replication cycle in the presence of a helper virus [17]. In the absence of a helper, however, AAV genomes can establish latency and persist as episomes [18] or in some cases integrate into host chromosomal DNA [19].

2.2. AAV-based vectors: properties and clinical success

Recombinant AAV vectors can be generated by replacing the endogenous *rep* and *cap* genes with an expression cassette consisting of a promoter driving a transgene of interest and a poly(A) tail (Fig. 1B). The *rep* and *cap* genes are then provided *in trans* as helper packaging plasmids together with adenoviral helper genes needed for AAV replication [10]. Over 100 natural AAV variants have been isolated, and variations in amino acid sequences result in somewhat different tropisms (the range of cells and tissues a virus can infect) [20], though none are pathogens [21]. Recombinant vectors have been generated from a number of these serotypes [10], though vectors based on AAV-serotype 2 (AAV2) have been the most widely studied and used in preclinical models and clinical trials to date. In general, vectors based on natural AAV variants have desirable gene delivery properties: a lack of pathogenicity and immunotoxicity, which grants them a strong safety profile [21]; the ability to infect dividing and non-dividing cells with reasonable efficiency [22]; the ability to mediate stable, long-term gene expression following delivery [20]; a ~5 kb genome that can carry a broad range of cargoes [23]; access to faster expression kinetics when using self-complementary, double stranded DNA forms of the vector genome [24]; and importantly the potential for engineering and optimizing the viral capsid and thus vector delivery properties [15]. Accordingly, AAV-based vectors have been harnessed in an increasing number of clinical trials (>130 to date) for tissue targets including liver, lung, brain, eye, and muscle [10,25]. As a result of its properties, as mentioned above, AAV has enabled clinical efficacy in an increasing number of trials for monogenic diseases [5,26–29].

For oncology applications, AAV vectors can transduce a wide variety of cancer primary cells and cell lines [30–32] and have the capacity to carry highly potent therapeutic payloads for cancer including anti-angiogenesis genes, suicide genes, immunostimulatory genes, and DNA encoding smaller nucleic acids (*e.g.* shRNAs and siRNAs) for post-transcriptional regulation of oncogenes [33]. AAVs therefore offer a strong potential as gene delivery vehicles for cancer gene therapy and have consequently been employed in numerous preclinical cancer models and in early stage clinical trials for cancer.

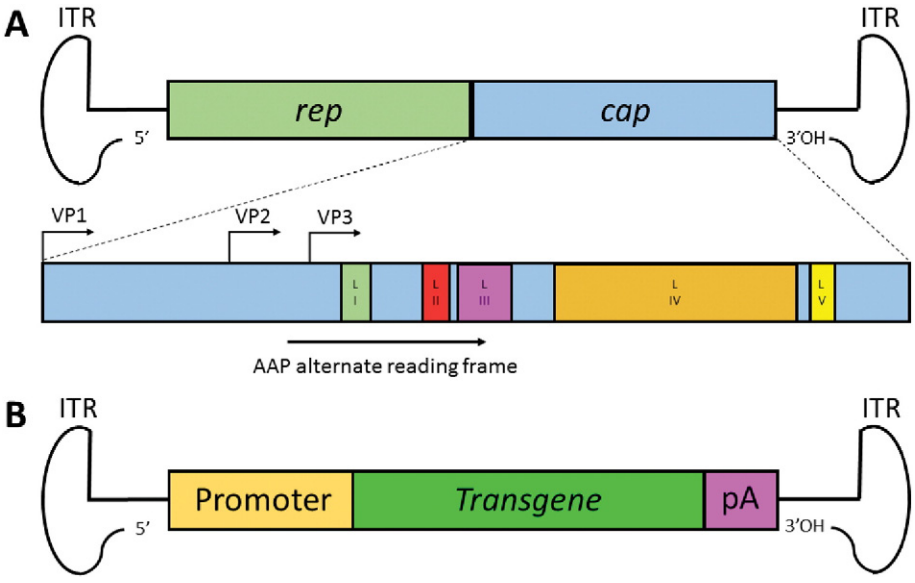

Fig. 1. Genomic structure of AAV and AAV vectors. (A) The 4.7 kb AAV genome is composed of the *rep* and *cap* genes flanked by inverted terminal repeats (ITRs). The *rep* gene codes for non-structural proteins involved in viral replication, packaging, and genomic integration, while the *cap* gene encodes the structural proteins VP1, VP2, and VP3 that assemble to form the viral capsid in a ratio of 1:1:10, respectively, in a total of 60 protein subunits; the assembly-activating protein (AAP) is translated from an alternate open reading frame. Also depicted are loop domains I through V (LI-LV), which contain variable regions that influence gene delivery properties. (B) Recombinant AAV vectors are generated by replacing the *rep* and *cap* genes with a gene expression cassette (*e.g.* promoter, transgene, and poly(A) tail) flanked by the ITRs. Vectors are then packaged by supplying the *rep* and *cap* genes *in trans* as well as adenoviral helper genes required for AAV replication.