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Utilizing Adeno-Associated Virus Vectors for Advancing Cancer Gene Therapy

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Abstract. Adeno-associated virus (AAV) has proven highly effective as a delivery vector in clinical trials for gene therapy. The safety and efficacy of AAV have led to its application as a novel approach for cancer treatment. Many successful clinical trials of cancer have demonstrated the feasibility of this approach. This review mainly introduces the structure, characteristics and application of AAV in tumor models. The promise of gene therapy in addressing diverse diseases has become evident, with AAV vectors playing a pivotal role in its achievements. AAV's exceptional safety profile and efficacy in gene therapy have spurred their application as a novel approach in the realm of cancer treatment. Clinical trials in this area have yielded impressive results, underlining the potential of AAV in combating cancer. This review provides a comprehensive insight into AAV's structural attributes and unique characteristics, emphasizing their significance in the context of tumor models. AAV's ability to efficiently deliver therapeutic genes to cancer cells, coupled with minimal risk, marks a significant breakthrough in oncology.

Keywords: Adeno-associated virus; gene therapy; cancer treatment; tumor models.

1. Introduction

Cancer is characterized by uncontrolled cell growth in the body, leading to its spread to other regions, and it stands as one of the diseases associated with a significant mortality rate. According to a report from 2020, approximately 10 million people worldwide succumbed to various diseases [1]. Cancer has consistently posed a significant global public health challenge. Conventional treatment methods, such as chemotherapy, often entail certain adverse side effects for patients [2]. Consequently, there is an imperative demand for a novel and efficient treatment approach that minimizes the negative impact on patients.

Gene therapy is the pursuit of modifying or manipulating gene expression or changing the biological characteristics of living cells for therapeutic intentions. It has found applications in diverse medical conditions, including cardiovascular diseases and immune system disorders, among others. According to statistics, as of 2015, there were at least 2,335 completed or ongoing gene therapy clinical trials worldwide [3]. Through a multitude of research endeavors and experiments, gene therapy has emerged as a viable and promising treatment modality.

Gene therapy involves delivering genes into cells often relies on a crucial tool known as a "delivery vector" for transportation. However, the development of a safe and effective delivery vector has consistently posed a significant challenge in the realm of gene therapy. These vectors can be broadly categorized into two groups: non-viral and viral vectors.

Non-viral vectors are characterized by their lower cytotoxicity, immunogenicity, and mutagenicity, as well as their ease of mass production, editing, and modification [4]. Nonetheless, their transfection efficiency is suboptimal, and they exhibit a higher degree of non-specific targeting.

Conversely, viral vectors have been engineered to be safe, with humans intentionally deleting the genes responsible for replication and assembly in these viruses, ensuring that viral vectors effectively infect cells without replication [5]. Moreover, their toxicity is minimal, and after undergoing a purification process to eliminate impurities, viral vectors have a negligible impact on the physiological function of the infected cells. These vectors contain marker genes that facilitate the assessment of virus infection efficiency and the screening of stable cell lines. However, optimizing

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the vector system for these viruses presents a formidable challenge, underscoring the complexity of their use in clinical trials of gene therapy. Currently, the most frequently employed viral vectors include adenovirus, lentivirus, and adeno-associated virus [6].

AAV stands out as one of the most successful and promising vectors in the field of gene therapy. Its efficacy in gene delivery, lack of pathogenicity, high safety profile, ability to target a wide range of host cells, and long-lasting in vivo expression have contributed to its success in numerous clinical trials [7].

For instance, in the treatment of hereditary deafness, AAV-based gene therapy using AAV-Otoferlin has demonstrated remarkable results. Both in newborn and post-cochlear development Otoferlin null mice, this therapy has led to significant hearing recovery, with the effects persisting for more than six months.

In another example, AAV vectors have been utilized for gene transfer in a mouse breast cancer xenograft model, focusing on the HSV/TK gene. This approach resulted in reduced tumor growth, showcasing the potential of AAV vectors in cancer treatment.

Due to its exceptional gene delivery capabilities, numerous preclinical models and clinical trials have utilized AAV vectors, which hold significant promise in cancer therapy [8].

In this review, the evolution of AAV will be delved into, exploring its structural characteristics, underlying mechanisms, and the challenges it presents in the field of gene therapy.

2. AAV and AAV vectors

AAV has a genome size of 4.7 kb and is a single-stranded DNA parvovirus. As shown in Fig.1, it consists of several key components, including an inverted terminal repeat (ITR) sequence and intermediate rep and cap genes. The ITRs play a pivotal role in viral replication and packaging processes [9].

The rep gene is responsible for encoding non-structural proteins that play essential roles in viral replication, packaging, and genome integration. VP1, VP2, and VP3 are structural proteins encoded by the cap gene, on the other hand. Nested within the cap gene is an additional open reading frame that encodes the assembly activator protein AAP, which plays a pivotal role in targeting and assembling capsid proteins.

Recombinant AAV vectors are constructed by substituting the native rep and cap genes with an expression promoter that governs a transgene of interest, in conjunction with a poly(A) tail. (Fig.1) The rep and cap genes required for vector packaging are typically supplied by a packaging plasmid during the vector production process [9, 10].

It is noteworthy that more than 100 natural AAV variants have been identified, and their tropism are determined by slight differences in their amino acid sequences. Importantly, none of these variants are associated with pathogenicity. Recombinant vectors have been developed based on several of these serotypes, with AAV-serotype 2 (AAV2) being extensively studied and utilized in preclinical models and clinical trials. These natural AAV variants possess favourable gene delivery characteristics, including non-pathogenicity, minimal immunogenicity, the ability to efficiently infect both dividing and non-dividing cells, sustained gene expression post-delivery, and a genome size of approximately 5 kb that accommodates a wide range of genetic payloads. Additionally, when using self-complementary, double-stranded DNA forms of the vector genome, they exhibit faster expression kinetics. These vectors are continuously optimized to enhance their delivery properties.

As a result, AAV-based vectors have been increasingly employed in over 130 clinical trials targeting various tissues such as the liver, lung, brain, and muscle [11]. Their aforementioned properties have contributed to clinical efficacy in an expanding number of trials, particularly in the treatment of monogenic diseases. In the field of oncology applications, these vectors demonstrate their capacity

to efficiently transduce a wide spectrum of primary cancer cells and cell lines. This potential enables the delivery of exceptionally potent therapeutic payloads for combating cancer.

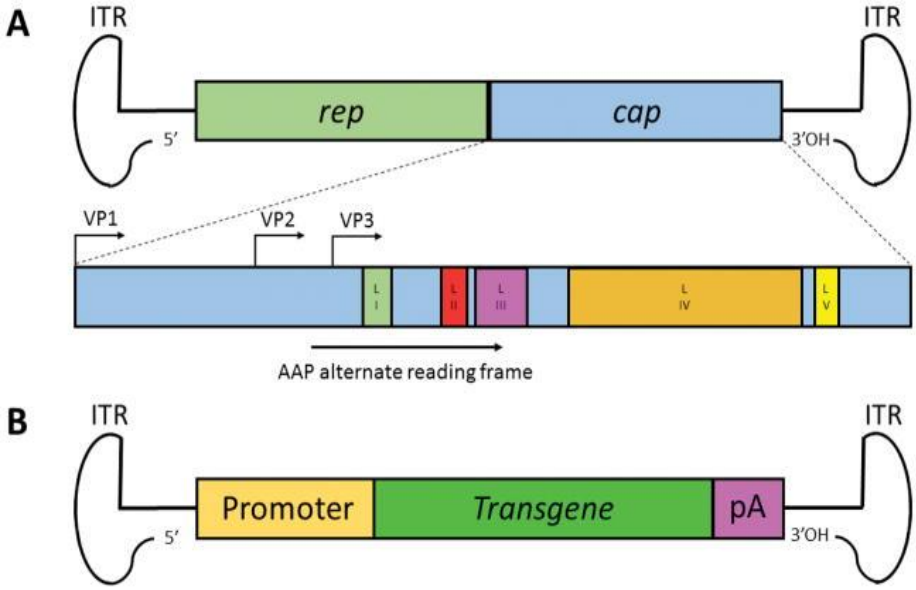

Figure 1. AAV(a) and recombinant AAV (b) architecture. [9]

3. Engineering AAV and delivery

Human clinical trials in the field of gene therapy have witnessed remarkable progress, largely attributed to the discovery of natural variants of AAV. These discoveries have significantly advanced the field. However, as researchers delve deeper into both basic research and clinical applications, they have become increasingly aware of certain limitations associated with these natural AAV serotypes, which constrain the full potential of recombinant AAV (rAAV) in gene therapy [12].

One of the key limitations stems from the tissue tropism exhibited by natural serotypes. This tropism, while useful in many cases, lacks the necessary specificity for a wide range of clinical applications and tends to have a broad infection range. Consequently, it falls short in achieving the precision required for targeted gene therapy.

Another challenge arises from the presence of neutralizing antibodies in the human body, which can impede the clinical use of AAV vectors. These antibodies may neutralize the vectors before they can effectively deliver their therapeutic cargo, limiting the success of AAV-based therapies in some cases.

Furthermore, certain natural serotypes may not meet the stringent requirements for high titer and purity necessary for clinical applications. These limitations have prompted scientists to undertake efforts aimed at developing more efficient AAV capsids. These engineered capsids are designed to enhance their ability to transport and transduce target cells, improve potency, and achieve selective expression of the transgene [13]. These advancements are crucial for overcoming the challenges posed by natural AAV variants and unlocking the full potential of AAV-based gene therapy.

The delivery properties of an AAV vector, including its interactions with tissue, vasculature, immune system components, target cell receptors, endosomal network, cytosol, and nucleus, are intricately determined by the amino acid sequence of the proteins comprising the viral capsid. Consequently, the modification of the AAV capsid through genetic engineering can yield novel variants with enhanced delivery characteristics [14]. These strategies bifurcate into two primary approaches: rational design, grounded in structure-function relationships to guide specific alterations, and directed evolution, which entails mutagenesis techniques to generate libraries of AAV capsids subjected to selective pressures for desired properties. Rational design tactics include modifying tyrosine residues to phenylalanine residues to mitigate proteasomal degradation and boost gene expression. Additionally, this approach has been instrumental in crafting variants that exhibit resistance against pre-existing

neutralizing antibodies by strategically mutating surface residues to evade recognition by Immunoglobulin G antibodies (IgGs) [15]. Rational design has also yielded variants capable of heightened transduction in tumor cells by integrating peptides with motifs that selectively bind to receptors highly expressed in cancer cells.

The path of AAV delivery, from administration to reaching target cells, is highly intricate and is often characterized by a lack of understanding of viral structure-function relationships that are essential for rational design. An alternative approach is founded on the idea that evolution can create new and valuable biological functions. Directed evolution has been used to create AAV variants that are significantly improved and tailored for different applications. Through various molecular techniques this methodology involves introducing genetic diversity into the AAV cap gene and generating extensive libraries of novel AAV varying. These libraries experience selective pressure to attain beneficial delivery characteristics. Several rounds of selection lead to the emergence of distinct AAV variants for therapeutic gene delivery in disease models. Directed evolution has effectively produced optimized AAV vectors with improved delivery capabilities, even in previously resistant cells, including epithelial cells and neural stem cells, both in laboratory environments and living organisms. Additionally, AAV vectors have undergone evolutionary modifications that enable them to more effectively disseminate within tissues and infect hitherto non-permissive cell types. In addition, in vivo directed evolution strategies may result in the creation of new AAV vectors that are ready for efficient gene delivery to tumors.

In pursuit of achieving full viral transduction, strategically placed short sequences containing negatively charged amino acids were inserted near the heparin binding domain of the AAV2 capsid. These sequences act as 'locks,' disrupting the virus and are bordered by protease cleavage sites that are recognized by matrix metalloproteinases (MMPs). The modified vectors equipped with these 'locks' displayed a reduced affinity for heparin and decreased infectivity. However, subsequent treatment with MMPs restored the ability to bind heparin, thereby facilitating efficient transduction. Importantly, the incorporation of these protein modifications has the potential to enhance selectivity toward cancerous tissues, primarily owing to the heightened expression levels of MMPs in most cancer types compared to normal tissue.

4. AAV-Mediated Delivery of Therapeutic Agents in Preclinical Cancer Models

AAV is seeing growing use in the context of introducing genes into preclinical in vivo tumor models. In the last ten years, AAV vectors have been utilized to transport various categories of genes, each serving unique functions. These include antiangiogenic genes, cytotoxic or suicide genes, immune-stimulating cytokines, tumor growth inhibitors, and antineoplastic genes designed to impede signaling pathways.

4.1. Therapeutic Strategies Targeting Angiogenesis

Angiogenesis inhibition therapy, which focuses on impeding the creation of fresh blood vessels from pre-existing ones (known as angiogenesis), plays a pivotal role in thwarting tumor nutrition, growth, and metastasis. As such, inhibiting tumor angiogenesis is a well-established approach in the fight against cancer, with the aim of diminishing the growth potential and metastatic capability of tumors. Angiogenesis is greatly influenced by vascular endothelial growth factor (VEGF)[16]. Numerous strategies have been devised for gene delivery to inhibit VEGF, including the use of decoy receptors, monoclonal antibodies, and gene delivery in conjunction with small-molecule inhibitors. For instance, Mahendra et al. employed AAV2 as a vehicle to deliver a soluble VEGF receptor 1 splice variant (sFlt1), functioning as a decoy that competitively interfered with the binding of VEGF-A to its endogenous receptor [17].

4.2. Transporting Genes for Tumor Suppression and Repair

Another successful approach in cancer gene therapy involves the delivery of transgenes and nucleic acids that can induce tumor suppression or reduce the expression of tumorigenic proteins, particularly those excessively produced in cancer cells. [18]. Numerous research teams have achieved success. For instance, survivin, an anti-apoptotic protein, can be countered effectively with dominant negative mutants. In a noteworthy study, Tu et al. utilized AAV2 as a delivery vehicle to introduce the C84A survivin mutant, which possesses the capacity to initiate apoptosis, into SW1116 and Colo205 colon cancer cells [19]. Subsequently, these genetically modified cells were subcutaneously injected into nude mice, leading to a remarkable 80% inhibition of tumorigenesis and a significant 55% reduction in tumor growth [20].

5. Conclusion

Due to their exceptional safety record and efficient gene delivery capabilities, AAV vectors have become a cornerstone in clinical settings. At present, more than 130 clinical trials are employing AAV vectors to address a diverse range of conditions in various tissues. The European Union's approval of Glybera and the successful experimental results of AAV vector gene therapy in treating congenital deafness in mice underscore the substantial potential of AAV-mediated therapeutic gene delivery. These advances also foreshadow the imminent progress and regulatory authorization of AAV gene therapies in the United States.

This review highlights the extensive use of AAV vectors, particularly AAV2, to transport a diverse array of transgenes in various preclinical cancer models. These strategies encompass factors that impede blood vessel formation, genes that induce cell death, genes that bolster the immune response and generate antigens, genes that curtail tumor growth, and monoclonal antibodies. Although gene therapy clinical trials targeting cancer treatment are widespread, the adoption of AAV vectors in this context is a relatively recent development. Historically, research predominantly revolved around oncolytic viruses such as adenovirus, herpes simplex virus, and reovirus, with non-viral approaches receiving relatively less emphasis. However, there are several advantages offered by AAV vectors that can be effectively harnessed for anti-cancer therapies. The potential for highly efficient transduction rates, the ability to engineer vectors for targeted delivery, and gene expression within cells that no longer divide are some of the advantages.

Looking toward the future, further advancements in AAV vectors hold the potential to enhance their suitability as gene delivery vehicles for cancer treatments. Developing innovative vectors that have a specific affinity for targeted tissues, minimal Non-specific or unintended transduction., and the ability to avoid antibodies that are already present in the body before treatment. would facilitate elevated levels of gene expression, which is crucial for achieving a robust therapeutic effect. Optimizing AAV vectors for localization in both primary and secondary tumors, as well as tumor-initiating cells that often exhibit resistance to conventional therapies and significantly contribute to unfavorable prognoses and post-treatment relapses in numerous cancers, would significantly enhance their efficacy. Vectors can be tailored for specific transduction via various administration routes, including systemic delivery, localized injection into non-tumor tissue, or intratumoral application. The choice of administration method may impact the efficacy of gene transfer. Directed evolution has shown promise in improving vector characteristics and creating novel AAV vectors for cancer treatments. By combining capsid engineering with advanced payloads, tissue selectivity, robust expression, and optimized genomic capacity can be attained to enhance efficient transgene delivery.

In addition to the aforementioned discoveries, the combination of AAV gene transfer and chemotherapy or alternative treatments has demonstrated potential in the treatment of diverse cancers. This strategy enhances the efficacy of therapy and improves patient outcomes. Notably, it has exhibited remarkable success in overcoming drug resistance by sensitizing resistant cancer cells and reinstating their susceptibility to treatment. The amalgamation of gene delivery with conventional therapies amplifies tumor targeting while minimizing off-target effects. Precise gene delivery reduces

harm to normal tissues while specifically focusing on tumor cells. Gene therapy holds promise for personalized medicine as it tailors treatments based on each patient's unique molecular profile. Relevant research in the field of cancer holds great promise by combining AAV gene delivery with conventional therapies. This integration not only enhances the efficacy of treatments but also opens up avenues for personalized medicine, ultimately benefiting patients. The ongoing investigation into innovative approaches against tumors will significantly contribute to our comprehension and control of cancer.

References

[1] B.S. Chhikara, K. Parang, Chem. Biol. Lett., 10, 451 (2023).
[2] C. Sonigo, I. Beau, N. Binart, M. Grynberg, Int. J. Mol. Sci., 20, 5342 (2019).
[3] E. Hanna, C. Rémuzat, P. Auquier, M. Toumi, JMAHP., 5, 1265293 (2017).
[4] H. Zu, D. Gao, AAPS J., 23, 78 (2021).
[5] J.N. Warnock, C. Daigre, M. Al-Rubeai, Methods Mol. Biol., 737, 1(2011).
[6] D. Bouard, N. Alazard-Dany, F.-L. Cosset, BRIT J PHARMACOL., 157, 153 (2009).
[7] Y. Qu, Y. Liu, A. F. Noor, J. Tran, R. Li, Neural Regen Res., 14, 931 (2019).
[8] M.F. Naso, B. Tomkowicz, W. L. Perry, BioDrugs, 31, 317 (2017).
[9] J.L. Santiago-Ortiz, D.V. Schaffer, J Control Release, 240, 287 - 301 (2016).
[10] D. Wang, P. W. Tai, G. Gao, Nat. Rev. Drug Discov., 18, 358 (2019).
[11] J. F. Wright, Gene Ther., 15, 840 (2008).
[12] C. Li, R. J. Samulski, Nat. Rev. Genet., 21, 255 (2020).
[13] J. L. Santiago-Ortiz, D. V. Schaffer, J Control Release, 240, 287 (2016).
[14] Z. Wu, A. Asokan, R. J. Samulski, Mol. Ther., 14, 316 (2006).
[15] R. Waehler, S. J. Russell, D. T. Curiel, Nat. Rev. Genet., 8, 573 (2007).
[16] F. Shojaei, Cancer Lett., 320, 130 (2012).
[17] S. Rafii, D. Lyden, R. Benezra, K. Hattori, B. Heissig, Nat. Rev. Cancer, 2, 826 (2002).
[18] P. Trang, J. F. Wiggins, C. L. Daige, C. Cho, M. Omotola, D. Brown, F. J. Slack, Mol. Ther., 19, 1116 (2011).
[19] Z. Xue, P. H. Sun, L. M. Zhu, S. H. Jiang, M. M. Qiao, A. L. Chi, S. P. Tu, Oncol. Rep., 25, 1039 (2011).
[20] S. Fumoto, S. Kawakami, M. Hashida, K. Nishida, Gene Ther., 3 (2013).