The Mechanism of Adeno-associated Virus (AAV) Gene Therapy

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Abstract. Adeno-associated Virus (AAV) vector has become an important tool for vivo gene therapy due to its high specificity, low immunogenicity and long-term expressibility. There are multiple novel pharmaceuticals utilizing recombinant AAV vectors to treat genetic defect disease or disease related to gene expression disorder that are undergoing clinical trials. In this review, the basic biological features of AAV viruses are introduced, including their structure, classification, and interactions with hosts. This paper aims to elucidate the commonalities and differences among several AAV viruses used in clinical treatment by analyzing existing literature. The relevant genes for transduction of various types of AAV viruses are specifically analyzed in this article. By elaborating the process and mechanism of AAV virus transduction-related gene expression, this paper also attempts to summarize the current achievements of AAV therapy and explain the limitations still existing. It is hoped to contribute to the development and progress of AAV therapy in the future.

Keywords: AAV vectors, gene therapy, mechanism, serotype.

1. Introduction

Genetic mutations or abnormal gene expression will cause humans to suffer from incurable diseases that have complicated clinical symptoms. Emerging and rapidly developing gene therapies may be able to achieve fundamental treatment or long-term prevention of certain diseases by modifying relevant nucleic acid sequences or correcting the transcription and translation process of specific genes. Among them, Adeno-associated virus (AAV) has been used as an important virus vector for in vivo gene therapy, which has undergone extensive trials to evaluate its safety and efficacy based on its different types, showing good safety, tolerability, and efficacy characteristics, with promising clinical application prospects [1].

The Adeno-associated virus was first identified and separated in preparations of a simian adenovirus (AdV) around 1965 [2]. Extensive observation and analysis by relevant researchers determined the basic biological information, structural features and specific classification of AAV, providing reliable scientific basis for its subsequent use as an important virus vector in gene therapy. The Adeno-associated virus belongs to the Parvoviridae family, which is a dependent parvovirus genus. AAV is a virus without a capsid, surrounded by a twenty-faced protein shell on the outside, and contains a single-stranded DNA genome of about 4.7-kb inside. The genome is flanked by two T-shaped inverted terminal repeats (ITRs) at the ends that largely serve as the viral origins of replication and the packaging signal [3]. Between ITRs, there are mainly three genes, Rep (Replication) gene, Cap (Capsid) gene and AAP (Assembly activating protein), which are responsible for encoding proteins needed for virus replication, encoding for the capsid and provides a scaffold for capsid assembly, respectively [4]. AAV is widely found in various vertebrates, including humans and non-human primates (NHPs) [3]. AAV has not been found to be pathogenic and can not replicate itself independently. It can only replicate itself when it coexists with helper viruses such as adenovirus, human papillomavirus and herpes virus [5]. Due to differences in the Cap gene and the proteins it produces, wild-type AAV is primarily divided into 13 serotypes (AAV1-AAV13) and has been detected with over 108 capsid variants. Each AAV serotype has its unique receptor and tissue specificity [6]. Different capsid variants can affect activation of cytotoxic T cell responses and clinical efficacy [7]. The choice of AAV serotype and capsid variant determines the affinity of AAV for

different cells and its distribution in the body, which finally determines the success of gene transfer or gene therapy.

Given the safety and specificity of AAV, it becomes a potential candidate for clinical use. By genetic engineering, an artificial recombinant AAV (rAAV) is developed. The rAAV lacks Rep gene, Cap gene and AAP, retaining only the ITRs, which reduces the immune response during gene delivery. Meanwhile, due to the obvious decrease in expression efficiency of rAAV with longer genome length [8], removing all the viral coding sequences can maximize the capacity for vector packaging and delivering. Because different types of AAV have different affinity for cells, rAAV is considered to have high specificity. Numerous clinical trials have already confirmed that rAAV can achieve localized specific gene therapy. Here, the mechanism of Adeno-associated Virus (AAV) gene therapy will be discussed by listing some typical AAV gene therapy methods.

2. The Mechanism of AAV Gene Therapy

2.1. Adeno-associated Virus (AAV) 2 gene therapy

Adeno-associated Virus (AAV) 2 is the earliest serotype that has been widely studied and used. In the 1990s, researchers had already conducted fundamental studies on AAV2, including isolation, cloning, and genome sequencing [9]. Subsequent extensive research has shown that AAV2 has excellent target specificity and tissue penetration, making it a promising candidate for gene therapy applications, such as the repair of retinal cells or liver cells. Currently, the initial designed AAV2 serotype is still the most commonly used in clinical studies and has the most safety and efficacy evidence, with numerous trials completed [1].

The single-stranded genome of AAV2 is only 4,675 nucleotides long and contains a terminal repeat sequence of 145 nucleotides [9]. The short genetic code length makes it easier to artificially reassemble it through genetic engineering, but it also limits the length of drugs or genes that can be delivered. AAV2 primarily targets the liver, skeletal muscle, nerves, and the retina of the eyes. In these tissues, the virus genome is widely distributed and protein expression levels are higher [10]. AAV2 utilizes three cell receptors: heparan sulfate proteoglycan (HSPG), AVβ-5 integrin, and fibroblast growth factor receptor 1 (FGFR-1). HSPG functions as the primary receptor, while the activities of the latter two receptors are comparable. As a cell surface receptor, HSPG can mediate AAV2 attached to the cell membrane to enter the cell through endocytosis. Then, AAV2 uses endosomes for intracellular transport. After escaping from late endosomes, AAV2 transfers to the nucleus and sheds its protein coat [3]. Single-stranded DNA must be converted into double-stranded form before it can be replicated or transcribed. After the expression of the Rep gene, the AAV genome is replicated and the Cap gene is expressed, completing the synthesis of the capsid. Finally, the assembly and release of the AAV virus for the next generation is achieved.

Recombinant AAV2 (rAAV2) is a specific gene-edited AAV2 virus that will serve as a key vector to carry and deliver relevant genes into cells to change the expression of specific proteins. RAAV2 is engineered to incorporate tissue-specific promoters, target genes, and poly A in place of Rep and Cap genes. This modification ensures specific expression of the target gene in particular tissue cells while minimizing the likelihood of integration into the host genome. In terms of selecting the target gene, it is possible to supplement missing genes according to needs or introduce gene fragments to inhibit the expression of the original genes. Similarly, gene editing technology can be used to correct the original gene sequence without changing the number of genes to restore its normal expression [11]. In the practical design of rAAV vectors, researchers often incorporate specific introns, enhancers, and other regulatory elements to modulate the expression level of the target gene [6].

In current clinical studies, due to the specific infection of AAV2 in retinal cells, many emerging local treatment drugs for visual disorders use the structure of rAAV2 vector plus deleted gene. Recently, two gene therapy drugs for the treatment of Leber hereditary optic neuropathy (LHON) that are currently in clinical development and have received partial approval from some countries - LUMEVOQ (GS010) and NR082, have adopted the structure of rAAV2 vector plus deleted gene.

GS010 uses rAAV2 vectors to deliver a normal human mitochondrial NADH-dehydrogenase subunit 4 (ND-4) gene with a specific mitochondrial targeting sequence (MTS) into cells [12]. After the rAAV2 is delivered into the cells, the ND-4 gene successfully transcribes into corresponding mRNA, which is then transported to the ribosomes on the surface of the mitochondria, promoting the expression and transfer of the missing protein in the mitochondria and improving the visual ability of LHON patients. NR082 also contains the ND-4 gene. It is a new type of rAAV2 ophthalmic injection that is currently undergoing clinical trials to evaluate its safety and effectiveness. Besides, Timrepigene emparvovec is a drug that is currently undergoing clinical trials, using the rAAV2 vector and a missing CHM gene structure for the treatment of Choroideremia (CHM), a disease caused by retinal degeneration [13, 14].

Due to the fact that different serotypes of capsid proteins can also affect the target specificity and tissue penetration of rAAV vectors [7], rAAV2 vectors with different capsid serotypes are often used to generate hybrid virus vectors (rAAV2/N, where N represents different capsid serotypes).This hybrid virus not only has the stable expression and gene integration ability of AAV2 type, but also has tissue specificity and different transduction efficiencies in the same type of tissue cells [15].

2.2. Adeno-associated Virus (AAV) 5 gene therapy

Adeno-associated Virus (AAV) 5 belongs to the Adeno-associated virus B (AAV1-4, 6-13 belong to Adeno-associated virus A) which was successfully isolated from human condyloma acuminatum on the penis and its transcription pattern is different from that of AAV2 [16, 17]. Meanwhile, the capsid assembly of AAV5 is not dependent on the AAP gene [17]. Because AAV5 differs significantly from other AAV serotypes and has a lower seroprevalence, it may improve the problem of reduced gene transduction efficiency due to the high preexisting AAV2 neutralizing antibodies in the human body, providing a more diverse range of treatment options. AAV5 mainly utilizes sialic acid as its cell receptor. The activity of Platelet-derived growth factor re-ceptor (PDGFR) is less than that of sialic acid. AAV5 primarily targets retinal cells, respiratory epithelial cells, vascular endothelial cell and liver cell. Because there are more sialic acid receptors on the surface of the liver, AAV5 is more commonly used in the treatment of liver diseases.

Roctavian (valoctocogene roxaparvovec) is the first gene therapy product approved by the FDA that is based on the rAAV5 vector and used to treat Hemophilia A, a genetic bleeding disorder caused by mutations in the gene that produces factor VIII (FVIII). The rAAV5 vector carries a genetic variant of the coagulation factor IX (FIX) called FIX-Padua, which can express the FIX coagulation factor in liver cells, secret it outside the liver cells and enter the bloodstream to perform coagulation functions, thus achieving the therapeutic effect [18]. Typically, a single dose is effective for a long period of time. Base on the high targeting to the liver of rAAV5, there are currently several clinical trials for treating different types of Hemophilia using rAAV5 vectors, such as AMT 061 (Hemophilia B) and BBM-H803 (Hemophilia A). AMT-061 (Etranacogene dezaparvovec) also chose to connect the rAAV5 vector with the FIX Padua variant and add a liver-specific promoter to regulate the expression of the target gene.

Despite the significant success of rAAV2 as a vector in the treatment of eye diseases, especially those caused by gene mutations in the retina, using rAAV5 as an alternative serotype can address more complex host and vector-related immune challenges. AAV5-DCN and AAV5-PEDF are a combination gene therapy that is currently being studied using the rAAV5 vector to eliminate corneal fibrosis and form new blood vessels [19].

2.3. Adeno-associated Virus (AAV) 8 gene therapy

Adeno-associated Virus (AAV) 8 was initially isolated through PCR technology from tissue samples of rhesus macaques, rather than being isolated as a complete and live virus like AAV1-6 [20]. AAV8 utilizes 37/67-kDa Laminin receptor (LamR) as its cell receptor. AAV8 primarily targets cardiomyocytes, retinal cells, liver cells and kidney cells. Related studies have shown that AAV8 has a higher affinity for liver cells than AAV2, and its transduction efficiency in liver cells is much higher

than that of AAV2, allowing for sustained high expression [20, 21]. AAV8 also has a superior delivery rate for cardiac transduction compared to AAV9 [22]. Its higher transduction efficiency may solve the adverse reactions caused by high doses of AAV2. In addition, AAV8 has excellent tissue specificity, with a particularly strong affinity for cardiomyocytes and hepatocytes, making it promising for delivering therapeutic target genes to specific tissues in the treatment of heart and liver diseases.

In the treatment of liver diseases, DTX401 is a drug for treating GSD-Iα (hereditary glycogen storage disease) that uses the rAAV8 viral vector to deliver the defective gene to liver cells and is currently undergoing clinical Phase III trials. By fusing rAAV8 with a gene that governs G6Pase-α enzyme, capitalizing on AAV8's liver cell targeting ability, the target gene is introduced into the patient's hepatic cells, leading to stable and efficient expression, thereby enhancing G6Pase-αenzyme activity, reducing hepatic glycogen levels and regulating blood glucose.

In the treatment of heart diseases, AAV8 can deliver either missing genes to restore normal expression or suppressive genes to achieve gene silencing. Related researchers have used rAAV8 vectors to deliver a gene encoding Urocortin-2 into cardiac cells [23]. After successful expression of the Urocortin-2 gene, an improvement in the function of the failing heart was observed [23]. AAV8 can also deliver small interfering RNA (siRNA) or short hairpin RNA (shRNA) to heart cells, specifically inhibiting overexpressed pathogenic genes and achieving gene silencing. For example, delivering Endothelin-1 shRNA via rAAV8 into the heart can lower hypertension caused by low temperatures [24].

3. Discussion

Through analyzing the application of AAV, includingAAV2, AAV5, and AAV8, which are common serotypes of AAV, as vectors in therapy, we can conclude that AAV is an emerging gene therapy with high targeting and long-term expressibility. It could provide long-term fundamental treatment for patients with rare genetic diseases by combing with gene engineering, gene editing etc. However, it is still noticeable that AAV gene therapy has certain limitations, such as limited capacity of the carrier, limited targeting of tissues by AAV, a relatively long time cycle for rAAV to induce therapeutic effects, the existence of pre-existing antibodies against AAV in the human body, the possibility of causing severe side effects, inconsistent purity and titers in large-scale production, and the unknown long-term effectiveness and safety of the treatment.

Limited capacity makes a large number of genetic diseases caused by multiple gene mutations excluded from AAV therapy. The limited tissue targeting of AAV restricts its applicability, posing challenges in drug design and gene therapy for diseases affecting other organs and tissues, such as the intestines. Related studies also have shown that IgG and neutralizing antibodies (NAbs) against AAV1 and AAV2 are widely prevalent in the population. These antibodies can bind to the AAV vector or inhibit subsequent AAV transduction, thus preventing target cells from binding to the AAV vector and lowering transduction efficiency, reducing therapeutic efficacy. In this situation, people often try to achieve the desired therapeutic effect by increasing the dose. However, in a large number of clinical observations, high-dose AAV gene therapy may trigger severe immune responses in patients, such as liver toxicity, cardiac toxicity, dorsal root ganglion toxicity, inflammatory reactions etc. These serious adverse reactions may cause fatal injuries or death to patients. Therefore, prior to treatment, attention should be paid to the detection and screening of NAbs in patients. In the future, efforts should be made to develop more efficient transduction vectors such as engineering AAV vectors, design capsid variants to evade vaccine system tracking or selectively inhibit the activation of B/T cells to reduce the level of neutralizing antibody induction, thereby achieving effective treatment at lower doses with lower immune response conditions.

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