Drug Discovery: Recent Progress and the Future

Review

Adeno Associated Virus (AAV) as a Tool for Clinical and Experimental Delivery of Target Genes into the Mammalian Retina

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Abstract

With an increasing number of identified causative genes, the widespread use of gene therapy is quickly becoming feasible. Once a target gene is selected, it is important to have a cell delivery method that is both specific and efficient. Cell type specificity and high efficiency is particularly important for the treatment of retinal degeneration, since viruses are efficient gene delivery vehicles for the nervous system, but often bring with them non-specific infections. In this review, we focus on adeno-associated virus (AAV). Over the last few decades, AAV has become a leading choice for safe gene delivery, in part due to its replication deficiency in cells without a helper virus. Here, we summarize the tropism of recombinant AAV (rAAV) for various types of mammalian retinal neurons in relation to capsid serotype and administration method. We also include our recent findings on an AAV serotype that AAV was specifically infected mouse cone photoreceptors when delivered by subretinal administration.

Key words mammalian retina; adeno associated virus; gene therapy

1. INTRODUCTION

AAV was initially discovered as a contaminant of an adenovirus stock preparation. Since AAVs are unable to replicate without helper virus co-infection, they are a useful tool for gene therapy in treating neurodegenerative disorders where further cell injury or death is to be avoided. Conveniently, AAVs can be handled at the biological safety level 1 (BSL1). The AAV genome contains three genes for Replication (Rep), Capsid (Cap) and Assembly (aap) that are flanked by inverted terminal repeats (ITRs), which are required for genome replication and packaging. The Rep gene encodes 4 proteins, Rep40, Rep52, Rep68, and Rep72, that are involved in viral replication. The aap gene encodes the assembly activating protein (AAP), which is required for capsid assembly, in an alternate reading frame within the Cap gene sequence. The Cap gene encodes the three structural proteins, VP1, VP2 and VP3, that make up the icosahedral capsid which mediates cell binding and internalization. Currently, there are known to be 13 AAV serotypes (AAV1–AAV13) which differ in the structures of their capsids. The serotypes have variable cell tropisms. AAV2, AAV3, AAV5 and AAV6 were obtained from human cells, whereas AAV1, AAV4, AAV7, AAV8, AAV9, AAV10, AAV11, AAV12 and AAV13 were from non-primate cells. For cell-specific gene delivery, researchers typically use the recombinant AAV2 genome but vary the capsid serotype into which it is incorporated.

2. THE RETINA AS THEAPEUTIC TARGET

Vision is one of the most important senses for our QOL. In addition, the retina is superficially located and easy to access in isolation from other organs. Hence, retinal diseases resulting from gene mutation are an attractive target for gene therapy. The retina contains five neurons types; photoreceptors (PR), bipolar cells (BC), horizontal cells (HC), amacrine cells (AC), and ganglion cells (GC), and one type of glia, the Müller cell (MG) (Fig. 1). The PRs first convert light into an electrical signal, and play an indispensable role in mediating form vision. The human retina does not have a uniform distribution of PRs. Instead, cone PRs, which mediate color and high acuity vision in bright light, are concentrated in the fovea. The fovea is devoid of rod photoreceptors, which become numerically dominant outside this central retinal region. An example of a retinal disease that has been recently addressed with gene therapy is Leber’s congenital amarosisis (LCA). LCA accounts for more than 5% of all inherited retinopathies and is estimated to affect 1 in 8100 to 1 in 30000 live births. LCA is one of the most severe forms of inherited retinal disease involving both rod and cone PRs, as well as the retinal pigment epithelium (RPE). The causative gene for LCA2 is RPE65, which encodes an RPE-specific 65kDa protein that functions as a retinoid isomerohydrolase to convert all cis-retinyl esters to 11-cis retinol following phototransduction. A phase 1 trial demonstrated the benefits and safety of delivering to LCA2 patients RPE65 cDNA by rAAV to the subretinal space. Gene therapy with RPE65 cDNA successfully improves visual acuity, as more recently demonstrated in a Phase 3 trial. LCA2 is preferable for gene therapy trials since the target gene is small and degeneration is slower, which leads to a favorable therapeutic window.
Other inherited diseases, such as Stargardt disease and Usher IB syndrome, are purely degenerative and caused by larger genes; therefore, AAV gene therapies for these are now under investigation and in clinical trials.22

3. TROPISM OF RECOMBINANT AAV IN THE MAMMALIAN RETINA AND AS AN EXPERIMENTAL TOOL FOR INFECTING SPECIFIC RETINAL CELL TYPES

Since genome sequences are highly conserved among the serotypes, the AAV2 genome is used as the basis for recombination and packaged with capsids from one of the 13 different serotypes to generate recombinant pseudotypes, such as AAV2/x (where ‘x’ is the number of the capsid serotype). So far only AAV2/2 has been utilized for clinical trials involving retinal gene therapy. The use of recombinant AAV (rAAV) as a gene delivery tool is limited by its ability to accommodate insert genes which, when flanked by ITRs, should have a single strand length not exceeding 4.7kb. If the recombinant gene size is larger than that of the primary AAV genome, viral production yields or transgene recombination are reduced.23,24

3.1. Specific Expression of Target Genes by the Use of rAAV2/2

Experimental studies show that rAAV serotypes have different tropisms in the mammalian retina. These differences can potentially be exploited to target cell delivery without specific promoters. Here we comprehensively summarize the data for rAAV serotype tropism in the mouse retina, and in other species to a limited extent, taking into account the location of the injection site, which may be either intravitreal or subretinal.

AAV2/2 is the most commonly studied serotype for gene transfer. AAV2/2 can infect various retinal cell types. In the mouse, subretinal administration—that is, injection between the PRs and RPE—of rAAV2/2 results in dominant infection of RPE cells and PRs, whereas intravitreal injection results in the infection of Müller cells and GCs (Table 1). In the ground squirrel, intravitreal injection of a capsid-mutated AAV2/2, in which 6 tyrosines are replaced by phenylalanines, produces selective infection of BCs.25 Further selectivity can be obtained by including a cell specific promoter, but promoters are not available for all retinal cell subtypes, and when promoters are included, this may cause the viral genome to exceed a length of 4.7kb. In the mouse, it is possible enhance selective expression by using promoter-driven Cre lines. The gene of interest is then flanked by loxP prior to incorporation into the rAAV. Using several Cre driver mouse lines, the expression of target genes into AC or GC types was successful.26 Enhanced neuron type selectivity can be obtained by breeding mouse lines in which two different promoters each drive the expression of a different recombinase, leading to a small intersectional population of neurons. This population can then be accessed by incorporating dual flanking into the rAAV.27

3.2. Differential Tropism of rAAV Based on Serotypes

rAAV2/1, 2/2, and 2/5 are the primary serotypes that have been examined for their tropism in the mouse retina. rAAVs were administered subretinally and intravitreally into adult mice, and rAAV2/5 were reported to be infected in the RPE and PRs. However, rAAV2/1 were restricted in the RPE by subretinal administration. rAAV2/2 transduced to the inner retina by intravitreal injection.28 After these initial studies in early 2000, detailed localization studies were eventually performed. Following postnatal injection of rAAVs day 7 (P7) into the retina subretinally, rAAV2/1 transduced to RPE, whereas rAAV2/2 and 2/5 transduced to RPE and PRs. rAAV2/2 efficiently transduced to the inner retina by intravitreal injection.29 A study in adult rats showed the results of intravitreal injection of rAAV2/1, 2/2, 2/3, 2/4, 2/5, 2/6, 2/7, and 2/8. With the exception of rAAV2/6, all serotypes showed a preference to transduce to GCL, whereas rAAV2/6 showed diverged infection profiles.30 Subretinal administration at postnatal day 0 mice were performed for rAAV2/1, 2/2, 2/5, 2/8, 2/9, 2/10 and 2/11. rAAV2/5 transduced PR cells and rAAV2/9 transduced most of the retinal layers except BC and MGs. rAAV2/1, rAAV2/2, rAAV2/8, rAAV2/9 and rAAV2/10 transduced HC3 and GCs.31 (Table 1)

3.3. Naive and Engineered rAAVs That Transduce to Cone PR

Previous reports suggest that rAAV2/6 transduces the RPE in the adult retina following subretinal administration but is not expressed in PRs.32 We recently tested for the expression pattern of AAV2/6 following subretinal administration in the adult mouse and found a different result. Namely, rAAV2/6 preferentially infected cone PRs. We also observed that rAAV2/6 infected rods and inner retinal cells, but the expression frequency was much lower than in cones (Hori et al., in preparation).

Recently, cone PR specific transduction by engineered rAAV with specific promoters was reported. rAAV2 was designed as a special delivery tool to transduce target genes into cone PR using cone arrestin promoters (mCAR), synthentic human red opsin promoters (PR2.1 and PR1.7), and an engineered variant of rAAV2, rAAV2-7m8, in which a heptamer peptide (LGETTRP) was inserted into a loop IV of AAV2 to enhance retinal transduction properties.33–35 rAAV2-7m8 exhibits high efficiency in infecting the inner retinal layer by intravitreal injection. Although subretinal administration is of concern regarding retinal detachment and subsequent retinal degeneration, rAAV2-7m8 peripheral subretinal administration
| Species  | Mouse (adult) | Mouse (P0) | Rat (adult) | Rat (P0) | Dog | Monkey |
|----------|---------------|------------|-------------|----------|-----|--------|
| RPE      | AAV2/1 (eff)  | AAV2/1 (pri) | AAV2/4 (res) | AAV2/1 (well) | AAV2/4 (res) | AAV2/4 (res) |
|          | (res)         | (eff)      |             | (well)   | (eff) | (eff)  |
|          | AAV2/2 (pri)  | AAV2/2 (well) |             | AAV2/2 (well) | AAV2/2 (well) | AAV2/2 (eff) |
|          | (eff)         | (well)      |             | (well)   | (eff) | (eff)  |
|          | AAV2/5 (pri)  | AAV2/5 (well) |             | AAV2/5 (well) | AAV2/5 (well) | AAV2/7 (eff) |
|          | (sca)         | (well)      |             | (well)   | (sca) | (eff)  |
|          | AAV2/6 (No other) | AAV2/6 (well) |             | AAV2/6 (well) | AAV2/6 (well) | AAV2/8 (eff) |
|          | (sca)         | (well)      |             | (well)   | (sca) | (eff)  |
|          | AAV2/8 (eff)  | AAV2/8 (well) |             | AAV2/8 (well) | AAV2/8 (well) | AAV2/9 (eff) |
|          | (eff)         | (well)      |             | (well)   | (eff) | (eff)  |
|          | AAV2/9 (eff)  | AAV2/9 (well) |             | AAV2/9 (well) | AAV2/9 (well) | AAV2/9 (eff) |
|          | (eff)         | (well)      |             | (well)   | (eff) | (eff)  |
| PR       | AAV2/2 (eff)  | AAV2/1 (Rod; 65.5%, Cone; 80.8%) | AAV2/2 (over 20%) | AAV2/2 (over 50%) | AAV2/2 (cone; about 30%) |
|          | (sca)         | AAV2/5 (Rod; 84.5%, Cone; 92.2%) | AAV2/5 (over 20%) | AAV2/5 (over 50%) | AAV2/7 (rod; eff, cone; about 40%) |
|          | AAV2/7 (eff)  | AAV2/9 (over 50%) | AAV2/7 (sca) | AAV2/7 (over 20%) | AAV2/8 (rod; eff, cone; about 20%) |
|          | (eff)         |             | AAV2/11 (Rod; 70.0%, Cone; 56.3%) | AAV2/11 (Rod; 84.5%, Cone; 92.2%) | AAV2/9 (cone; about 40%) |
|          | AAV2/8 (eff)  |             | AAV2/10 (rod; eff, cone; about 20%) | AAV2/10 (rod; eff, cone; about 20%) | AAV2/9 (cone; about 40%) |
|          | (eff)         |             | AAV2/11 (rod; eff, cone; about 20%) | AAV2/11 (rod; eff, cone; about 20%) | AAV2/9 (cone; about 40%) |
| HC       | AAV2/8 (eff)  | AAV2/1 (47.9%) | AAV2/2 (78.2%) | AAV2/2 (78.2%) | AAV2/4 (21%) |
| BC       | AAV2/6 (21%)  | AAV2/4 (about 20%) | AAV2/6 (22%) | AAV2/6 (22%) | AAV2/2 |
| MG       | AAV2/1 (some) | AAV2/1 (pri)  | AAV2/2 (over 40%) | AAV2/2 (over 40%) | AAV2/1 (over 50%) |
|          | (eff)         | (eff)       | AAV2/4 (about 20%) | AAV2/4 (about 20%) | AAV2/1 (over 50%) |
|          | AAV2/2 (eff, pre) | AAV2/2 (over 40%) | AAV2/6 (25%) | AAV2/6 (25%) | AAV2/2 |
| AC       | AAV2/8 (eff)  | AAV2/9 (34.8%) | AAV2/4 (about 20%) | AAV2/4 (about 20%) | AAV2/1 (over 50%) |
| GC       | AAV2/2 (eff, pre) | AAV2/2 (46.9%) | AAV2/2 (over 60%) | AAV2/2 (over 60%) | AAV2/2 |
|          | (eff)         | AAV2/2 (46.9%) | AAV2/2 (over 60%) | AAV2/2 (over 60%) | AAV2/2 |
|          | AAV2/8 (eff)  | AAV2/8 (40.7%) | AAV2/8 (over 50%) | AAV2/8 (over 50%) | AAV2/2 |
|          | (eff)         | AAV2/8 (40.7%) | AAV2/8 (over 50%) | AAV2/8 (over 50%) | AAV2/2 |
|          | AAV2/9 (eff)  | AAV2/9 (82.0%) | AAV2/9 (over 50%) | AAV2/9 (over 50%) | AAV2/2 |
|          | (eff)         | AAV2/9 (82.0%) | AAV2/9 (over 50%) | AAV2/9 (over 50%) | AAV2/2 |
|          | AAV2/10 (eff) | AAV2/10 (72.1%) | AAV2/10 (over 50%) | AAV2/10 (over 50%) | AAV2/2 |
|          | (eff)         | AAV2/10 (72.1%) | AAV2/10 (over 50%) | AAV2/10 (over 50%) | AAV2/2 |

We followed descriptions of efficiency to respective reports. eff; efficiently, res; restricted, pri; primarily, sca; scattered, pre; predominantly.
led to high therapeutic gene expression and visual acuity.\textsuperscript{35}

4. CONCLUSION

rAAV studies have significantly contributed to progress in the retinal studies for both clinical and experimental usage. To further restrict expression and to improve efficiency of rAAV, especially for use in human gene therapy, the trend is to select for cell-type specific promoters, to test and engineer the various capsid serotypes for enhanced tropism and expression levels, and to preferentially use intravitreal rather than subretinal administration. rAAV may further be employed for manipulating target genes via clustered regularly interspaced short palindromic repeats (CRISPR)-based genome techniques.\textsuperscript{36}

Conflict of Interest The authors declare no conflict of interest.

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