Adeno-Associated Viruses

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Adeno-associated viruses (AAVs) are a convenient tool for gene therapy delivery. According to the current classification, they are divided into the species AAV A and AAV B within the genus Dependoparvovirus. Historically AAVs were also subdivided on the intraspecies level into 13 serotypes, which differ in tissue tropism and targeted gene delivery capacity. Serotype, however, is not a universal taxonomic category, and their assignment is not always robust. Cross-reactivity has been shown, indicating that classification could not rely on the results of serological tests alone. Moreover, since the isolation of AAV4, all subsequent AAVs were subdivided into serotypes based primarily on genetic differences and phylogenetic reconstructions.

Keywords: adeno-associated virus ; AAV ; classification

1. AAV Biology

Adeno-associated viruses (AAVs) are non-enveloped particles with a size of 18–26 nm. Sixty protein molecules form an icosahedral capsid. The genome is represented by a linear single-stranded DNA of approximately 4.7 thousand bases.

The AAV genome contains two open reading frames (ORFs): (1) *rep*, which encodes four overlapping proteins necessary for the regulation of viral DNA replication: Rep78, Rep68, Rep52, and Rep40 [1][2]; and (2) *cap* encoding viral capsid proteins: VP1, VP2 and VP3 [3][4]. The genome is flanked by inverted terminal repeats (ITRs) [5], which form a T-shaped self-complementary secondary structure with a free 3′-hydroxyl that acts as a replication primer [6][7].

For effective replication and reproduction in host cells, AAV requires co-infection with an auxiliary virus, e.g., adenovirus (hence the name adeno-associated), although AAV replication is also possible with herpesvirus, cytomegalovirus, and papillomavirus co-infection [8].

The best-known hosts of AAVs are primates and humans [9][10], although these viruses have been found in other animals [11][12][13]. AAVs do not cause a significant immune response or any notable pathology in the host cells. Consequently, they are not considered pathogenic [4]. Notably, over 90% of the adult population is seropositive to AAVs, i.e., are likely asymptomatic carriers [14]. The prevalence of AAV in distinct tissues varies between 37 and 72% [15].

2. AAV Gene Therapy

AAVs are a convenient tool for gene therapy [16][17]. Since wild AAVs persist in the form of episomes (integration into the host genome at AAVS1 19q13.3-qter site is extremely rare [18] and absent in vectors), they are safer compared to retroviruses, which insert into the host genome randomly, leading to oncogenesis [19]. AAVs also have reduced immunogenicity compared to adenoviruses, further supporting their utility as gene therapy vectors [20].

Results of preclinical and clinical studies have demonstrated that AAV vectors are safe and effective tools in gene therapy for a range of diseases including cystic fibrosis, hemophilia B, arthritis, hereditary emphysema, muscular dystrophy, Parkinson’s disease, Alzheimer’s disease, prostate cancer, malignant melanoma, epilepsy, and others [16][21][22][23]. To date, three AAV-vector-based products have been approved for use in medical practice: Glybera (familial lipoprotein lipase deficiency) [24], Luxturna (hereditary retinal dystrophy) [25] and Zolgensma (spinal muscular atrophy) [26].

3. Current AAV Classification

Current AAV classification is based on the phenotype (the shape of the virion), replication peculiarities, and the host range [27]. According to these criteria, AAVs are represented by two species—AAV A and AAV B. Besides AAVs, there are eight other species within the genus dependovirus (ICTV: https://talk.ictvonline.org/taxonomy/ accessed 25 June 2021).

Additionally, AAVs are subdivided on the intraspecies level into serotypes. The first reports of serologically distinct AAVs date back to 1966, when the first three serotypes were identified [28].

Further, any newly identified AAV serologically distinct from known types were assigned a new serotype in chronological order. To date, 13 AAV serotypes are known. However, there are significant ambiguities and controversies in the properties and definitions of the AAV serotypes. For instance, AAV4 was identified based on the reaction with antiserum [29], whereas AAV5 was assigned based on the DNA structure differences identified by restriction analysis and blot-hybridization [30][31]. This protocol could correspond to the identification of a new genotype, which is consistent with the later assignment of AAV5 to a separate species within the dependovirus genus by the International Committee on Taxonomy of Viruses [32].

AAV6 was assigned a new serotype based on genetic differences from the complete genomes of AAV2 and AAV3 (82% identity), as well as AAV4 (75–78% identity) [33]. However, its cap genes were 96% similar to AAV1, and most likely AAV6 is a variant of AAV1. Despite this, AAV serotype 6 is still in use.

In 2002, during an investigation of the asymptomatic presence of AAV in primate tissues, Gao et al. identified AAV7 and AAV8 [34]. It was used signature regions—a fragment of genomic sequence at positions 2886–3143 nt unique to each AAV type (previously identified by Rutledge et al. [33])—to define distinct virus types, and named the newly isolated viruses according to the differences in these regions. The *rep* and *cap* nucleotide sequences and the predicted amino acid sequence comparisons for AAV1-8 (with the exception of AAV5 as obviously incongruent) revealed differences, primarily in the region encoding capsid proteins. AAV7 was shown to have a 63% to 85% similarity to the amino acid sequences and 68% to 84% of the nucleotide sequences of other AAV serotypes; similar results were obtained for AAV8. The serological difference of AAV7, AAV8, and other serotypes was also established based on the absence of neutralization by any antiserum other than their own (anti-AAV7 and anti-AAV8, respectively).

Later, Gao et al. identified 11 phylogenetic groups based on phylogenetic analysis of cap sequences—so-called ‘clades’—from A to F, consisting of phylogenetically similar representatives from three or more sources, and five groups of clones (phylogenetically similar representatives from less than three sources) [35].

AAV10 and AAV11 were also identified based on the signature region differences and characterized according to the cap sequence [36]. The serological analysis confirmed that AAV10 and AAV11 were serologically distinct from AAV2.

Similarly, AAV12 was identified based on the *rep* and *cap* sequence differences [37]. Finally, AAV(VR-942) was isolated in 2008 and had a high degree of amino acid sequence similarity of rep with AAV4 (98%) and AAV3 (93%), as well as 93% identity in VP1 as compared to AAV3. Despite this, AAV(VR-942) has a distinct pattern of cellular receptor interaction and was suggested as a new serotype, AAV13 (the name is currently present only in the corresponding GenBank entry); it should be noted that serological studies were not conducted for AAV13 [38].

Thus, since the isolation of AAV4, all subsequent AAVs were subdivided into different serotypes based primarily on genetic differences and phylogenetic reconstructions. In addition to the established 13 serotypes, one of which comprises a separate virus species (AAV5), more than 100 novel genetic variants (so-called serovars [39]) are not assigned to any taxonomic unit below the species level. It is unlikely that they will be all tested serologically, and the genetic boundaries of types are not robust.

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