Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Virology

In vivo tissue-tropism of adeno-associated viral vectors

Arun Srivastava[1,2]

In this review, a brief account of the historical perspective of the discovery of the first cellular receptor and co-receptor of the prototype adeno-associated virus serotype 2 (AAV2) will be presented. The Subsequent discovery of a number of AAV serotypes, and attempts to identify the cellular receptors and co-receptors for these serotype vectors has had significant implications in their use in human gene therapy. As additional AAV serotypes are discovered and isolated, a detailed understanding of their tropism is certainly likely to play a key role in all future studies, both basic science as well as clinical.

Addresses
[1] Division of Cellular and Molecular Therapy, Department of Pediatrics, Powell Gene Therapy Center, University of Florida College of Medicine, 2033 Mowry Road, Gainesville, FL 32611, United States
[2] Department of Molecular Genetics & Microbiology, Powell Gene Therapy Center, University of Florida College of Medicine, 2033 Mowry Road, Gainesville, FL 32611, United States

Corresponding author: Srivastava, Arun (aruns@peds.ufl.edu)

Current Opinion in Virology 2016, **21**:75–80

This review comes from a themed issue on **Viral gene therapy vector-host interactions**

Edited by **David Markusic** and **Roland Herzog**

http://dx.doi.org/10.1016/j.coviro.2016.08.003

Introduction

Adeno-associated virus (AAV) is a small, naked icosahedral virus, which was first discovered in 1965 [1]. In addition to being a single-stranded DNA containing virus, AAV remains the only virus that has not been conclusively proven to be the etiologic agent of any human disease to date. On the contrary, recombinant AAV vectors have been used in a number of Phase I/II clinical trials, and in some cases, have shown clinical efficacy in the potential gene therapy of several human diseases [2••,3–5,6••,7–10•]. Although many of the steps in the life cycle of AAV have been studied extensively, details at the molecular level continue to emerge. In addition, in recent years, a number of additional AAV serotypes have been isolated, and their use as vectors is likely to further greatly expand the landscape for their optimal use for therapeutic purposes. In spite of these exciting developments, the molecular bases of the varied tissue-tropisms of the AAV serotype vectors have not been fully delineated. In this review, I will attempt to shed light on this aspect of AAV vector biology.

Discovery of the cellular receptor for AAV2

As stated above, AAV2 was discovered in 1965 [1]. However, because AAV2 tropism transcended the species barrier, the conventional wisdom for nearly three decades was that AAV2 infection was non-specific. In 1996, Ponnazhagan *et al.* [11•] identified the first human cell line that could not be infected by the wild-type AAV2, or transduced by recombinant AAV2 vectors, and suggested that AAV2 infection of human cells was receptor-mediated.

The search for the putative cellular receptor for AAV2 intensified. In 1996, Mizukami *et al.* [12] reported that a 150-kDa protein present in membranes could bind to AAV2, and suggested that it might be the cellular receptor for AAV2, but provided no corroborating evidence. In 1998, Summerford and Samulski [13•] identified heparan sulfate proteoglycan (HSPG) as the cellular receptor for AAV2. This provided an explanation for the wide tropism of AAV2 since all cells across the species barrier express HSPG, except for the first human cell type identified by Ponnazhagan *et al.* [11•]. The discovery of the cellular receptor for AAV2 also provided the explanation why the very first Phase I clinical trial performed by Flotte and colleagues [14•] for the potential gene therapy of cystic fibrosis, although established the safety of recombinant AAV2 vectors in humans, did not achieve clinical efficacy, even though that was not the primary objective. The elegant studies by Duan *et al.* [15] documented that HSPG is expressed predominantly on the baso-lateral surface, rather than on the apical surface, of primary human airway epithelial cells, and thus AAV2 vectors failed to efficiently transduce these cells.

Discovery of the cellular co-receptors for AAV2

Soon after the discovery of the cellular receptor for AAV2, it also became apparent that HSPG, which is required for binding of AAV2 to the cellular membrane, is not sufficient for the viral entry into cells. In 1999, Qing *et al.* [16•] identified the human fibroblast growth factor receptor 1 (FGFR1) as the first cellular co-receptor for AAV2. Simultaneously, Summerford *et al.* [17] also identified αVβ5 as yet another co-receptor for AAV2.

On the basis of these studies, a clearer picture emerged of the underlying mechanism of AAV2 binding and entry into target cells. However, Chen *et al.* [18] reported the isolation of AAV sequences from various tissues, predominantly

tonsils, from children, and showed that 7% of these 'AAV2-like' sequences shared ~98% identity with the wild-type AAV2. Interestingly, these AAV2-like viruses lacked the HSPG-binding site, and failed to bind to the cellular receptor. These studies suggested that either the use of HSPG as a receptor by AAV2 was a consequence of *in vitro* propagation of AAV2 in culture, or alternatively, AAV2 utilizes multiple putative cellular receptors. Indeed, recombinant AAV2 vectors lacking the HSPG-binding site have been shown to exhibit efficient and widespread transduction in murine brain and retinal tissues [19,20]. Similarly, in addition to FGFR1 and αVβ5, at least four additional cellular co-receptors, hepatocyte growth factor receptor (HGFR) [21], α5β1 integrin [22]; laminin receptor (LamR) [23]; and CD9 [24] have been shown to be utilized by AAV2 by as cellular co-receptors to date.

Discovery of additional AAV serotypes

Multiple AAV serotypes have been isolated from tissue culture stocks, humans, as well as non-human primates [25–29,30••,31–33]. Following their development as recombinant vectors, their efficacy has been evaluated in various tissue culture cell lines. To date, 13 distinct AAV serotype vectors (AAV1–AAV13) have been described, but this number is certainly likely to grow. In general, whereas AAV1–AAV6 serotype vectors transduce tissue culture cells to various degrees of efficiency, for the most part, AAV7–AAV13 serotype vectors transduce tissue culture cells poorly *in vitro*, but these serotype vectors efficiently transduce various tissues and organs in various animal models *in vivo*.

Although the precise mechanism of tissue-tropism of other AAV serotype vectors *in vivo* remains unknown, it has become increasingly clear that attachment to putative cell surface receptors is the initial step for successful transduction. It has also become clear that the attachment of most of the AAV serotype vectors is first mediated by binding to various cell surface glycans, which serve as primary receptors. To date, 23 different glycan receptors for AAV serotype vectors have been identified, such as: α2-3 and α2-6 N-linked sialic acid (SIA) for AAV1 [34,35]; HSPG for AAV2, AAV3, and AAV13 [13•,33,36]; α2-3 O-linked and α2-3 N-linked SIAs for AAV4 and AAV5, respectively [37–39]; HSPG and α2-3 and α2-6 N-linked SIA for AAV6 [35,40,41]; and termimal N-linked galactose (GAL) of SIA for AAV9 [42,43]. The primary cellular receptors for AAV7, AAV8, AAV9, AAVrh10, AAV11, AAV12, and AAV13 remain unknown. In general, AAV serotype vectors can be grouped into 3 categories with respect to their glycan receptor usage: HSPG for AAV2, AAV3, AAV6, and AAV13; SIA for AAV1, AAV4, AAV5, and AAV6; GAL for AAV9.

As with AAV2, binding to the primary cellular receptors is most likely not sufficient for AAV serotype vectors to gain entry into cells, and additional cell surface as co-receptors are required. The following cellular co-receptors identified thus far include: FGFR1 [16•], αVβ5 [17] and α5β1 [22] integrins for AAV2; a putative integrin for AAV9 [44]; FGFR1 for AAV2 [16•] and AAV3 [45]; hepatocyte growth factor receptor (HGFR) for AAV2 [21] and AAV3 [46]; platelet-derived growth factor receptor (PDGFR) for AAV5 [47]; epidermal growth factor receptor (EGFR) for AAV6 [48]; and laminin receptor (LamR) for AAV2, AAV3, AAV8, and AAV9 [23].

Following binding to the primary receptors, and interactions with the secondary co-receptors, AAV serotype vectors are internalized through endosomal pathways including clathrin-coated vesicles and/or clathrin-independent carriers/GPI-anchored-protein-enriched endosomal compartments (CLIC/GEEC) [49].

Discovery of AAVR

In 2016, using a genome-wide screen, Pillay *et al.* [50•] reported the identification of a trans-membrane protein, which was designated as an essential receptor for AAV2 infection (AAVR). AAVR was shown to bind directly to AAV2, and was capable of endocytosis of AAV from plasma membrane and trafficking to the trans-Golgi network. Deletion of AAVR rendered various mammalian cell types resistant to infection by AAV2. More interestingly, AAVR was found to be a critical factor for infection by several AAV serotypes, and AAVR-knockout mice were resistant to AAV infection. On the basis of these data, it was claimed that AAVR is a universal receptor for AAV infection, but it remains to be seen what role, if any, AAVR plays in large animal models, and especially in humans.

Animal models for AAV vector transduction

A large body of information has been gleaned from studies in mice, where different AAV serotype vectors have been shown to exhibit distinct tropism for various tissues and organs [51]. The efficacy of some of the AAV serotype vectors has also been evaluated in other animals, small and large, such as rats, gerbils, hamsters, rabbits, cats, dogs, horses, and non-human primates. For example, the first evidence of transduction by AAV2 vectors and long-term gene expression in the murine brain was reported by Kaplitt *et al.* in 1994 [52]. In 1996, first successful transduction of the mouse retina [53] and muscle [54] was reported. AAV2 vector-mediated gene transfer to the guinea pig cochlea [55] and to the primate lung [56] was also reported in 1996. In 1997, several groups first reported the transduction of the mouse liver [57,58•,59], and hematopoietic stem cells [60]. Successful transduction of the rabbit lung [61,62], and the rat carotid arteries [63] by AAV2 vectors was also reported in 1997. Subsequently, various AAV serotype vector-mediated gene transfer in various cell cells and tissues, such as intestinal epithelial cells [64], pancreatic beta cells [65], salivary glands [66], maxillary sinus [67] and temporomandibular joints [68],