



Recombinant AAV Vectors as Tools to Study and Treat Human Disorders

Magali Cucchiariⁿ*

Center of Experimental Orthopaedics, Saarland University Medical Center, Germany

Gene transfer, especially for the goal of human gene therapy, is the principle of administering therapeutic coding sequences in a target for sustained expression instead of providing a final product with short pharmacological half-life. The identification of genes associated with diverse human pathologies and the expansion of gene transfer vectors has allowed to develop a broad range of models of human disorders and to establish the first human clinical trials, to find applications at clinical levels for genetic disorders, neurologic and muscle disorders, cardiovascular diseases, infectious and other acquired diseases, for degenerative, chronic, inflammatory, or age-related diseases, and for tumors. The most common strategies are gene replacement therapies (gene targeting) or gene addition (growth and transcription factors, cytokines, and antagonists), the transfer of inhibitory nucleic acids, and the application of enhancers of immune responses (genetic vaccines). Evidence of the functionality of a gene transfer protocol necessitates an evaluation in culture systems *in vitro* prior to translation *in vivo* by either transplantation of cells genetically modified *ex vivo* or by direct administration of the candidate treatment *in vivo*. Direct approaches are simpler, as the cells are not manipulated before reimplantation, but indirect strategies permit a thorough characterization of the modified cells, without injecting viral particles, and are better suited when cell repopulation is an issue like for regenerative medicine.

Critical to the success of a gene transfer approach is the identification of a vector capable of both efficiently and durably expressing a transgene, often with a rapid onset and without being detrimental to the host. The systems used are based on either nonviral compounds (naked DNA, gene gun, electroporation, ultrasound-facilitated and hydrodynamic gene transfer, cationic lipid and polymer delivery) or on viruses (Ads: Adenoviruses; RVs: Retroviruses; HSVs: Herpes Simplex Viruses; AAV: Adeno-Associated Virus). Nonviral vectors are safe but only mediate transgene expression at low and short-term efficiencies [1,2]. Viral vectors are more potent, using natural entry pathways in the cell. Still, classical (Ads, RVs, HSVs) vectors have shortcomings (risk of insertional mutagenesis of RVs, immunogenicity of Ads, cytotoxicity of HSVs) [1,2] while recombinant AAVs (rAAVs) have emerged as favored gene vehicles. AAV is non-pathogenic, replication-defective human parvovirus (25 nm diameter, 4.7-kb single-stranded DNA) [3]. Most rAAVs derive from serotype 2 (AAV-2) [4] but others are now also available (AAV-1 to AAV-12) [5]. In generating rAAVs, both *rep* (replication) and *cap* (encapsidation) viral open reading frames can be deleted to maintain only the two inverted terminal repeats around the transgene cassette [6].

rAAVs have first been produced in the presence of a helper (Ad) virus that provides the *rep* and *cap* genes in trans but helper-free methods are now favored to avoid the risk of contamination by helper proteins, of unwanted immune responses to rAAV, and of creating replication-competent AAV particles by recombination [7-9]. Since, several reports have focused on the production of clinical-grade rAAVs to support clinical trials [5,10-12].

Permissivity to rAAV has been reported for both proliferating and quiescent cells (including progenitor cells) and in various tissues (muscle, bone, cartilage, soft tissues, synovium, skin, liver, brain and retina cells, lymphocytes/macrophages/monocytes, endothelial cells, etc.) [13-22] expressing the viral receptor (cell membrane-associated heparan sulfate proteoglycan, HSPG [23], and cell-specific co-receptors like the

FGF receptor 1, integrins $\text{tg } \beta_v\beta_3/\alpha_3\beta_1$, HGF receptor, PDGF receptor). Upon binding, rAAV is endocytosed and transported to the nucleus for uncoating and conversion to double-stranded DNA intermediates (circular and linear) [24]. As some tissues are refractory to transduction, knowing that intracellular trafficking of rAAV depends on surface (co-) receptor concentrations and on the biology of the serotype [25], large efforts have been made to bypass these rate-limiting steps for improved efficacy and specificity by modifying the capsid genes. New rAAV have been created by mutagenesis, chemical conjugation, peptide display libraries, or DNA shuffling and error-prone PCR, leading to mosaic, pseudotyped, chimeric, and hybrid (Ad/AAV, HSV/AAV) vectors. Advantage has also been taken of the hierarchy between serotypes over AAV-2 (AAV-1 for the muscle, AAV-5 for the brain, AAV-8 for the liver). Another issue has long been the limited capacity of rAAVs (~5 kb). This has been largely solved by using the ability of the virus to form head-to-tail (circular) DNA concatamers by intermolecular recombination [26-29]. Another important development in the use of rAAVs has been the generation of self-complementary rAAV (scAAV) to bypass the requirement of host cell-mediated rate-limiting synthesis of double-stranded DNA from the single-stranded rAAV genome [30]. Also notably, the wild-type AAV genome integrates specifically in the short 19p13-qter region of chromosome 19 (AAVS1 site), a process that only requires *Rep* proteins and the ITRs [31-33]. In the absence of *Rep* in the recombinant genome, rAAV transgenes persist as 99% of stable episomes actively and persistently transcribed (up to 1.5 years) [34,35] with only 1% of slow, nonspecific integrants, indicating a low risk of insertional mutagenesis.

All these properties have thus made rAAV a very powerful gene transfer system for *in vivo* applications [11,12,36-38] and the benefits of employing this class of vector in clinical protocols are under active investigation for cystic fibrosis [39-41] and α_1 anti-trypsin deficiency [42,43] for neurologic, retinal, and muscular diseases [36,44-56], for cardiovascular diseases [57-60], for viral infections and vaccine development [61], bone disorders [62], rheumatoid arthritis [63], and against malignancies [36].

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*Corresponding author: Magali Cucchiari, PhD, Associate Professor for Molecular Biology, Center of Experimental Orthopaedics, Saarland University Medical Center, Kirbergerstr. Bldg 37, D-66421 Homburg/Saar, Germany, Tel: ++49-6841-1624987; Fax: ++49-6841-1624988; E-mail: mmcucchiari@hotmail.com

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