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Adeno-associated virus and lentivirus vectors: A refined toolkit for the central nervous system

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ABSTRACT

The last two decades have witnessed the increasing instrumentalization of viruses, which have progressively evolved into highly potent gene transfer vehicles for a wide spectrum of applications. In the context of the central nervous system (CNS), their unique gene delivery features and targeting specificities have been exploited not only to improve our understanding of basic neurobiology, but also to investigate diseases or deliver therapeutic candidates. As a result, we have started moving away from the opportunistic use of recombinant vectors that are derived from naturally existing viruses towards the rational engineering of tailored lentivirus (LV) and adeno-associated virus (AAV) vectors for specific use in the CNS.

Keywords: **VIRAL VECTORS, LENTIVRUS, ADENO-ASSOCIATED VIRUS, CNS, GENE THERAPY, NEURODEGENERATIVE DISEASE, TRANSGENE EXPRESSION**

Highlights

- AAV and LV vectors for gene delivery in the central nervous system
- Gene therapy as a major driver for viral vector development
- Treatment of neurodegenerative diseases with gene therapies
- Viral vector-based modeling of diseases in animals
- Cell-type specific transgene expression
- Drug-inducible and optogenetic switches for spatial and temporal gene expression

VIRAL VECTORS

Virus-mediated gene delivery applications include fundamental neurobiological investigation [1], disease modeling [2], and gene therapy [3], each requiring a unique set of features: i.e. tropism, transduction efficiency, safety, and packaging capacity [4]. This has led to the investigation of more than a dozen viral species from various families [5]. Vectors derived from lentivirus and adeno-associated viruses are currently the most frequently used for rodent and primate CNS research and in gene therapy clinical trials [5,6].

Adeno-associated viral (AAV) vectors

AAVs are non-pathogenic, single stranded DNA viruses from the *Dependovirus* genus of the *Parvoviridae* that require helper virus infection for successful replication [7]. Viruses of this genus are non-enveloped and transduce dividing and non-dividing cells. A complete review of AAV biology is beyond the scope of this article but has been reviewed thoroughly elsewhere [8]. After transduction, the 4.7 kb viral genome remains predominantly episomal, providing long-term gene expression in non-dividing cells. The viral capsid is composed of three capsid proteins (VP1, VP2, and VP3) and the amino acids of the common VP region compose the protein domains that are exposed on the surface of the assembled capsid. They are responsible for surface topology and determine tropism and specificity [9]. Artificial AAV serotypes with new characteristics and tropisms have been produced by altering the cap genes [10]. The ease with which it is possible to alter AAV enables the development of large vector libraries (Figure 1) to screen and identify vectors with specific features that are tailored for specific applications [11].

Lentiviral vectors (LV)

Lentiviruses belong to the family of *Retroviridae.* The most extensively studied lentivirus is HIV-1 which possesses two copies of a positive sense RNA genome of

approximately 9 kb [12]. In contrast to gamma retroviruses, LV retroviruses have the unique ability to translocate across the nuclear membrane and infect non-dividing cells. The genome contains nine genes from which only the gag, pol, and rev genes are co-expressed for viral vector production in HEK293T host cells [13,14]. In this system, all virulence factors have been deleted to generate non-pathogenic and replication-deficient LV vectors [14]. The envelope gene of HIV-1 is, in most cases, replaced by a heterologous gene to alter tropism and specificity [15]. The most commonly used envelope is the vesicular stomatitis virus glycoprotein (VSV-G). LVs pseudotyped with the VSV-G efficiently transduce neurons (Figure 1) and are highly mechanically resistant, facilitating their concentration and purification [14].

APPLICATION OF AAV AND LV VECTORS FOR GENE THERAPY

The field of gene therapy has been the major driver for the research and development of viral vector technologies. The efforts are reflected by the 2356 clinical trials that have been conducted to date. Only 43 of these trials have targeted brain diseases (February 2016, http://www.wiley.com/legacy/wileychi/genmed/clinical/). The low number of CNS trials relative to other indications (cancer or immunodeficiency) highlights the difficulty of vector delivery to the CNS but also our only partial understanding of these diseases. The blood-brain barrier (BBB) limits entry of molecules to the brain. Thus, vectors are delivered directly to their site of action, most commonly via intracranial or intrathecal injection. Specific viral vectors are capable of crossing the BBB, but intravenous injection exposes the vectors to circulating antibodies, leads to widespread transduction of various tissues, and therefore requires very high doses of vector [16-18].

Due to the increased need of vectors for translating gene therapy applications into the clinic, the development of new protocols for viral vector production is becoming central as manufacturing protocols must be compliant with good manufacturing practice (GMP)

and suitable for the production and qualification of large batches. For a detailed review on this topic, please refer to the following publications [19,20].

Most clinical trials for treatments of neurodegenerative diseases used AAV or LV vectors (http://www.wiley.com/legacy/wileychi/genmed/clinical/). This includes AAV gene therapy for Canavan disease, a pediatric leukodystrophy [21], Parkinson's and Alzheimer's disease [22] [23], AADC deficiency [24] , and LV gene therapy for X-linked Adrenoleukodystrophy [25] and Metachromatic Leukodystrophy [26]. For a detailed review on gene therapy and clinical trials, refer to Choudhury et al. [3]. The therapeutic effects observed for most early clinical trials were limited, but more recent trials have shown very encouraging results [27]. The demonstration of safety, including the absence adverse effects resulting from insertional mutagenesis, has been an important finding [28], in addition to the therapeutic outcome. These early studies with first generation vectors were definitely a milestone, but they have also highlighted shortcomings and challenges, which have to be addressed to create improved vectors for future applications [27].

NEXT GENERATION OF VIRAL VECTOR-BASED GENE DELIVERY

The tropism and transduction pattern of a vector depends on multiple parameters, including its diffusion properties, the expression of receptors on target cells, the affinity of capsid or envelope proteins for the receptors, and intracellular factors. LV has a diameter of 100 nm and the transduced area in the parenchyma is limited to a few millimeters [29], whereas the diffusion of AAV (20 nm diameter) is highly dependent on each serotype [30-32]. Recent studies have focused on evaluating new AAV serotypes or pseudotyped LV vectors to identify the most potent vectors and take advantage of retrograde transport properties. Various AAV serotypes are capable of retrograde transport to distal neuronal projection sites (Figure 2) [33,34]. Retrograde transport of LV is obtained using specific envelopes of the Rhabdoviridae family [35,36] further increasing the versatility of these vectors in the CNS (Figure 1 and 2) (for comprehensive reviews see [12,37]).

Advances in vector technology are clearly critical for basic studies of the CNS as well as clinical development of therapeutic strategies, but other hurdles need to be overcome to fully translate basic research into the clinic. Applying vectors to targeting the brain in the CNS presents a considerable challenge regarding scale-up, as the human brain is approximately 3000 times larger than that of a mouse, the most commonly used animal model in research. This becomes especially problematic when the aim is to deliver the transgene to large regions of the CNS for therapies targeting Alzheimer's disease, lysosomal storage disorders, or Parkinson's disease [38]. Gene transfer via intra-parenchymal injections translates into a large number of injection tracts due to a diffusion distance of millimeters to a few centimeters in the brain parenchyma [39]. Convection-enhanced delivery of vectors to the brain parenchyma is one promising strategy to improve vector diffusion [40,41]. This technique has been frequently applied in studies on non-human primates [42] as well as recent clinical trials, and improves diffusion throughout the brain by maintaining the injection pressure at a level sufficient to overcome the hydrostatic pressure of the interstitial fluid [41,43].

Intravascular (IV) administration may be a very attractive alternative strategy. IV injection is non-invasive and could allow transduction of the entire brain due to the high capillary density of the CNS [44]. Potential disadvantages of this approach are possible clearance by circulating antibodies and inefficient penetration of the CNS through the BBB [18]. A compromise between IV- and IC-based approaches is the administration of vectors into the cerebrospinal fluid (CSF), which maximizes CNS exposure [38]. Studies in non-human primates have reported stronger transgene expression throughout the cortex and cerebellum after injection of an AAV9 vector into the cisterna magna relative to IV injection [18].

A strategy that utilizes a therapeutic gene that is secreted from the transduced cells combined with the above-described methods for administration could result in highly pervasive distribution throughout the CNS for a global therapeutic effect [45]. Transduction

of ependymal cells lining the ventricles after CSF injection for the expression of a factor in the CNS is one example of how this strategy could be applied [45].

In contrast to strategies that aim for global delivery to the CNS and make use of ubiquitous promoters for high transgene expression, some clinical studies, as well as disease modeling and fundamental studies, require controlled transgene expression, limited to just one specific cell-type, to investigate its function within a complex neuronal network or its relevance in pathology (Figure 1 and 2). Current efforts use cell-type specific promoters, microRNA target (miRT) sequences, and highly specific vectors (Figure 2) [32,46,47]. The miRNA belongs to the group of non-coding RNAs, which play a fundamental role in processes such as chromatin remodeling, gene expression, and transcript processing. The international ENCODE Consortium is currently cataloging functional DNA elements in the human genome in various cell types and tissues to shed light on regulation of gene expression [48,49]. Insights from this line of research will ultimately make it possible to precisely control gene expression in target cells after viral vector transduction. Such control of gene expression is important for studying the basic biology of the CNS and also to increase the safety of future therapeutic applications [32]. Currently, inducible promoters, or a combination of cell-type specific promoters coupled with inducible elements, allow good spatial and temporal control of transgene expression [50]. Drawbacks of drug inducible systems are leakiness, induction kinetics, and immune responses which have precluded clinical implementation of these systems [51].

Precise spatial and temporal resolutions are required to monitor neurobiological functions and to investigate synaptic connections of neuronal subpopulations in specific compartments of the CNS. Recent optogenetic approaches that couple viral vectors, light inducible proteins, and promoters specific for a neuronal phenotype are a powerful tool in neurosciences [52]. This approach allows precise spatial, temporal, and phenotype specific transgene expression after light induction at a given wavelength using optical fibers, and has been increasingly applied to CNS studies.

Altogether, the combination of the vector features, delivery strategies, neuronal circuitry, properties of the therapeutic candidates (secreted vs intracellular), and technologies to control transgene expression offer numerous opportunities for next generation gene delivery.

PERSPECTIVES

A viral vector toolkit with improved vectors that are tailored to specific applications is becoming increasingly realistic and is taking shape due to interdisciplinary research and the development of enabling technologies in the fields of virology, molecular biology, neurosciences, medicine, and engineering. Coupled with these new and powerful technologies, such as optogenetics and genome editing, viral vector based gene delivery will enable us to further increase our understanding of neurobiology. Furthermore, it facilitates the investigation of the molecular basis of pathologies and allows accurate modeling of neurological diseases. More refined viral vectors and transgenes will ultimately lead to a deeper understanding of the CNS and its diseases, which will allow the successful treatment, not only of neurodegenerative diseases, but also other indications associated with the CNS.

Highlights

- AAV and LV vectors for gene delivery in the central nervous system
- Gene therapy as a major driver for viral vector development
- Treatment of neurodegenerative diseases with gene therapies
- Viral vector-based modeling of diseases in animals
- Cell-type specific transgene expression
- Next generation viral vector-based delivery

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FIGURES

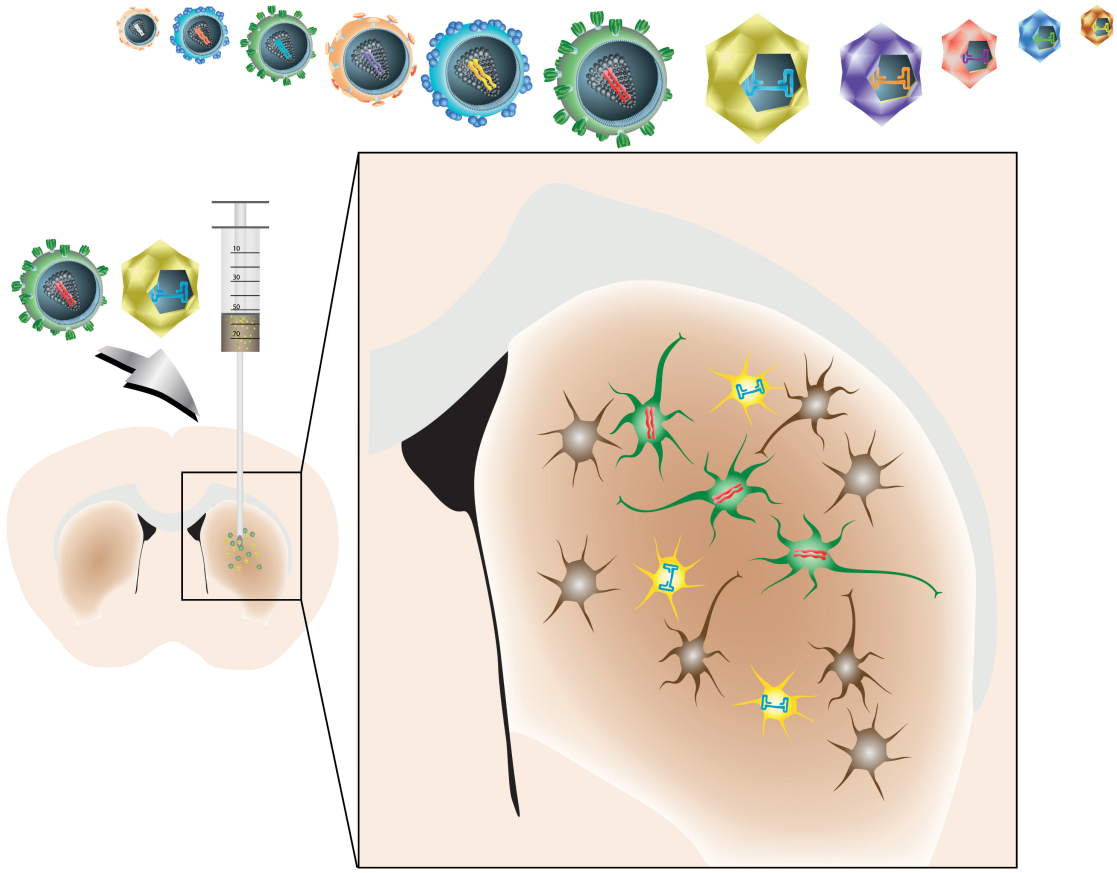

Figure 1

Cell-type specific targeting of CNS cells with various AAV serotypes and lentiviral vectors.

The scheme illustrates the large collection of AAV and lentiviral vectors available for CNS applications. Transduction of subpopulations of cells is made possible (neurons, astrocytes, microglial cells, plexus choroïd, progenitor cells, etc.) by modifying the capsids (AAV) or envelopes (LV), or by integrating specific regulatory elements (promoter, microRNA-based detargeting strategies).

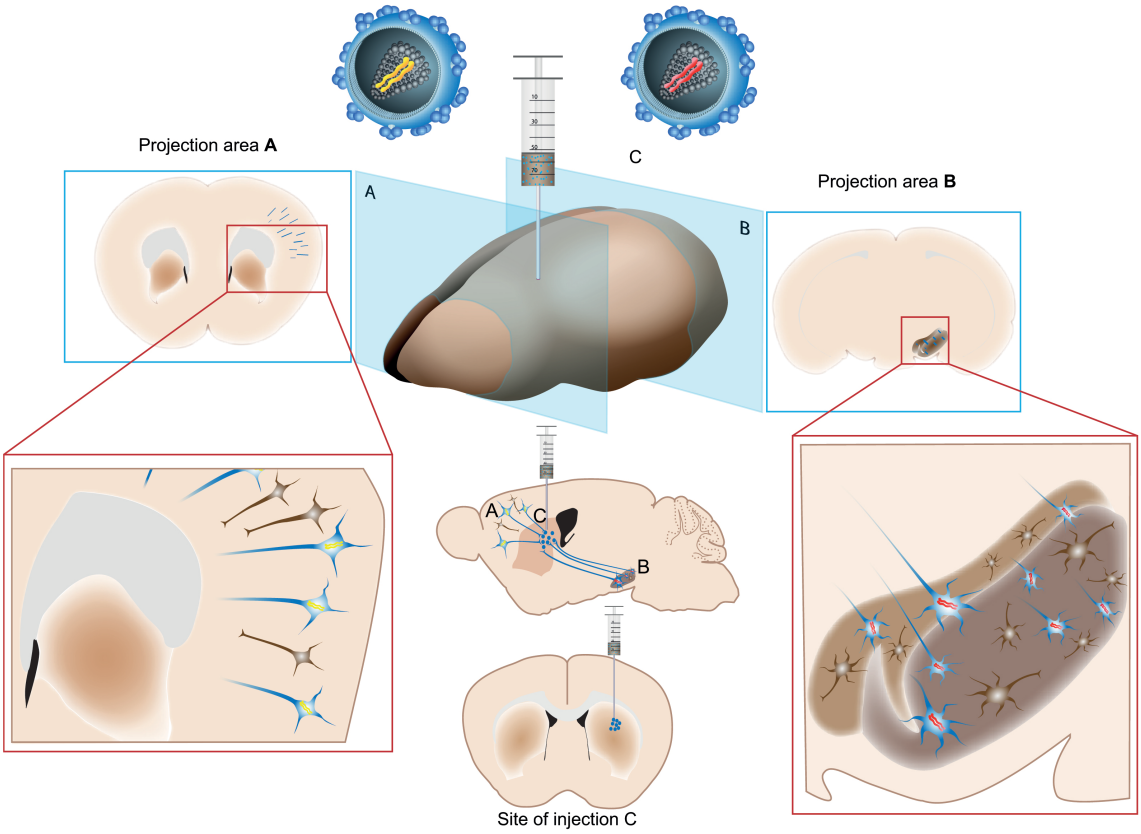

Figure 2

Scheme illustrating how retrograde transport properties and specific regulatory elements could be used to maximize gene transfer and/or target specific subpopulations of neurons in the brain.

LV vectors peusdotyped with envelopes of the Rhabdoviridae family infect cells at the injection site and are retrogradely transported to distal projection sites. The scheme illustrates a scenario in which transgene expression is cell-type specific. After transport to the cortex (Projection site A), the transgene exhibits expression of the yellow marker, whereas the expression of the red marker is limited to neurons in the substancia nigra (Projection site B).

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