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## Gene delivery with viral vectors for cerebrovascular diseases

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### Abstract

Recent achievements in the understanding of molecular events involved in the pathogenesis of central nervous system (CNS) injury have made gene transfer a promising approach for various neurological disorders, including cerebrovascular diseases. However, special obstacles, including the post-mitotic nature of neurons and the blood-brain barrier (BBB), constitute key challenges for gene delivery to the CNS. Despite the various limitations in current gene delivery systems, a spectrum of viral vectors has been successfully used to deliver genes to the CNS. Furthermore, recent advancements in vector engineering have improved the safety and delivery of viral vectors. Numerous viral vector-based clinical trials for neurological disorders have been initiated. This review will summarize the current implementation of viral gene delivery in the context of cerebrovascular diseases including ischemic stroke, hemorrhagic stroke and subarachnoid hemorrhage (SAH). In particular, we will discuss the potentially feasible ways in which viral vectors can be manipulated and exploited for use in neural delivery and therapy.

### Keywords

Gene Delivery; Viral Vectors; Lentiviral Vector; HSV; Adenoviral Vector; AAV; Cerebrovascular Diseases; Cerebral Ischemia; Review

## 2. INTRODUCTION

Gene transfer provides a powerful approach not only for the study of physiological processes but also for the treatment of various diseases. Gene therapy is a rapidly developing area, and is considered one of the most promising approaches for a variety of inherited and acquired disorders that currently have no alternative remedy. Ongoing gene therapy clinical trials target first and foremost cancer (1); however, neurological disorders, including cerebrovascular disease, are now a valid target. The CNS, in contrast to other organs, faces special obstacles in terms of gene delivery and therapy. For example, mixed cell populations that are highly intermingled in the brain complicate targeted therapeutic strategies. Furthermore, neurons, the major target population in the CNS, are post-mitotic and thus

present a twist in the utilization of viruses, which are often dependent on mitotic stages for effective transduction. Finally, and perhaps the most limiting for any brain-targeted therapeutic thus far, the CNS is isolated by the BBB (2). Despite the various limitations in current gene delivery systems, a spectrum of viral (and non-viral) vectors has been successfully used to deliver genes to the CNS. This review will focus primarily on systems that incorporate viral vectors, and the implementation of viral gene delivery in the context of cerebrovascular diseases including ischemic stroke, hemorrhagic stroke and subarachnoid hemorrhage (SAH). In particular, we will discuss the various ways in which viral vectors can be manipulated and exploited for use in neural delivery and therapy.

### 3. VIRAL VECTOR-BASED GENE DELIVERY INTO THE CNS

#### 3.1. Lentiviral vectors

Lentiviruses, a subclass of retrovirus, have acquired additional important features rendering them highly attractive as gene therapeutic vectors in brain. Perhaps most importantly, lentiviral vectors are able to infect both mitotic and post-mitotic cells, such as glia and neurons, respectively. Furthermore, lentiviruses possess an ability to translocate across the nuclear membrane and stably integrate into chromosomes, allowing them to mediate long-term gene expression while producing only a minimal immune response (3). These positive features have earned lentivirus-derived vectors a position as one of the principal gene delivery candidates for the CNS, leading to the initiation of a recent clinical trial using a lentiviral vector to treat Parkinson's disease (4).

The first generation of lentiviral vectors was derived from the human immunodeficiency virus 1 (HIV-1) with substantial modifications. The vector was made by co-transfection of a packaging plasmid encoding the viral genes (except for the env and vpu), an envelope plasmid encoding vesicular stomatitis virus glycoprotein protein (VSV-G) in place of the HIV-1 env proteins, and a transfer vector encoding the HIV-1 cis-acting elements, which enable the transgene to be encapsidated into the viral particles (5). This HIV-based viral vector was found capable of mediating stable reporter gene expression in neurons in rat brain (5). In order to further minimize the risk of triggering host immune responses and producing replication-incompetent recombinant viral particles, second and third generations of lentiviral vectors were developed by removing additional viral sequences or separating genes encoding the structural and other components required for packaging the viral genome onto three or four plasmids (6–8). Lentiviral vectors can accommodate up to 16 kb proviral length; however the viral titers are remarkably reduced when the viral genomic size increases (9). To obtain usable titers, the maximal packaging size of lentiviral vectors is estimated to be approximately 11 kb (9). These replication-deficient, self-inactivating vectors lead to long-term expression of transgenes with minimal immune response and inflammation, and provide neuroprotective effects in various models of CNS diseases (10–13). In addition to HIV-1-based lentiviral vectors, vectors derived from alternative lentivirus, such as equine infectious anemia virus (EIAV), simian immunodeficiency virus (SIV) and feline immunodeficiency virus (FIV), have also been developed for gene delivery to the CNS (14–16). Since these viruses in their native forms are non-pathogenic to humans, EIAV-,

SIV- and FIV-based vectors theoretically offer the advantage of biosafety for use in human gene therapy.

In order to improve tropism, viral vectors can be pseudotyped by modifications to the envelope proteins. The VSV-G envelope protein is widely used for pseudotyping lentiviral vectors due to its high stability and the potential to generate high-quality and high-titer stocks (17). However, several limitations exist with VSV-G-pseudotyped lentiviral vectors. First, and perhaps foremost, the VSV-G protein has been shown to be cytotoxic (17). Second, although VSV-G-pseudotyped vectors provide a broad tropism toward mitotic and post-mitotic cells, such as neurons and glia (5, 18), they do not undergo retrograde transport, an important mechanism to mediate the entry into the CNS from nerve endings of various viruses such as herpes and rabies (19, 20), and to mediate high-efficiency foreign gene delivery to the CNS via peripheral injection of recombinant viruses. As an alternative to pseudotyping with the VSV-G protein, lentiviral vectors can be pseudotyped using other envelope proteins, such as murine leukemia virus (MuLV), rabies virus and rabies-related virus envelope proteins. These alternatively pseudotyped lentiviral vectors exhibit reduced cytotoxicity and are capable of transducing CNS cells *in vitro* and *in vivo* (21, 22). Furthermore, rabies virus glycoprotein-pseudotyped lentiviral vectors preferentially transduce neurons, undergo retrograde transport and can be expressed in distal neurons *in vivo* after peripheral delivery (22–24). These traits have led to the exploration of gene therapy based on a rabies-pseudotyped vector in a model of motor neuron disease (25).

Lentiviral vectors also have great promise for *ex vivo* gene therapy due to their property of integration of viral genome into host genomes and their ability to mediate stable gene transduction of cell lines and primary cells. In this paradigm, lentiviral vectors are used to efficiently transduce neural stem/progenitor cells *in vitro* (26), and these are then developed as a delivery vehicle of therapeutic genes. NSCs modified with lentiviral vectors carrying various genes have been explored as a treatment in different models of CNS diseases (19, 27, 28).

Overall, lentiviral vectors offer stable and long-term transgene expression in CNS cells, particularly in post-mitotic neurons, with minimal immune response (5). These features allow them to be a highly attractive tool for *in vivo* and *ex vivo* gene therapy for CNS diseases. A lentiviral vector-based clinical trial for Parkinson's disease is currently underway (4). Further studies are focusing on novel non-integrating lentiviral vectors that are deficient in integrase activity. This modification may minimize the risk of integration-induced oncogenic mutations (29, 30). With increased biosafety, the application of lentiviral vectors in the clinical setting may become more prominent in the near future.

### 3.2. Adenoviral vectors

Adenoviral vectors were one of the first viral vectors employed for gene transfer into the CNS. As opposed to lentivirus, adenovirus cannot integrate into the host genome and therefore mediates transient, rather than stable, gene expression (31). However, adenoviral vectors are relatively easy to make and can be purified and concentrated up to  $10^{12}$  to  $10^{13}$  viral particles/ml, making them useful for gene therapy application. Adenoviral vectors are the most heavily used viral vectors in clinical trials for human diseases, with the majority of

studies focusing on the field of cancer gene therapy (32). However, no adenoviral vector-based clinical trial is currently underway for neurodegenerative diseases.

Adenoviruses contain five early genes (E1A, E1B, E2, E3 and E4) that are involved in viral replication. In adenoviral vectors, the regions of early adenoviral genes are usually replaced with an expression cassette containing the gene(s) of interest. The first generation of adenoviral vectors, which lack E1 and/or E3 and thus lose their ability to replicate, were found to elicit significant immune responses (even in the brain) due to pre-existing anti-viral immune responses (33–35). To avoid adenovirus-triggered immune response, the second generation of adenoviral vectors was generated by making additional deletions of E2 and/or E4 from the viral genome (36, 37). This generation increased cloning capacity compared to the first-generation vectors, but still did not completely avoid *in vivo*-associated immunogenicity due to the remaining viral genes. The third generation, also known as “gutless” adenoviral vectors, are devoid of all coding viral regions and contain only a packaging signal (38). In addition to minimizing the potential immune response (38, 39), these high-capacity adenoviral vectors can accommodate up to 36 kb of foreign DNA (38). Despite not incorporating into the host genome, “gutless” vectors achieve stable gene expression in the brain for up to one year (40, 41). Although these vectors still induce a capsid-mediated immune response in the rat brain (42), they display stability and efficiency in CNS gene delivery, especially for the delivery of larger molecular weight genes.

### 3.3. Adeno-associated viral (AAV) vector

AAV is a non-pathogenic virus and thus an attractive tool for human gene therapy. AAV vectors can transduce both dividing and non-dividing cells, can offer stable long-term expression, and can be generated at high clinical-grade titers (43). Thus, AAV is currently at the forefront of gene delivery for CNS diseases. Whereas adenoviral vectors are used predominantly in clinical trials for non-neural human diseases, AAV vectors have been applied the most to neurodegenerative diseases.

AAV is a single-stranded DNA virus. Its genome encodes two proteins, rep and cap. Rep is responsible for the replication, integration and rescue of virus, whereas cap encodes capsid structural proteins required for housing the viral genome. The rep-cap genome is flanked by inverted terminal repeats (ITRs), which are also required for the replication, rescue, integration and packaging of AAV (44). However, efficient replication of AAV genome and production of infective particles requires co-infection with a helper virus, such as adenovirus or herpes virus. After entering the host cell by receptor-mediated endocytosis, AAV engages in a lytic life cycle only in the presence of a helper virus. In the absence of helper functions, AAV genomes integrate into the host chromosome in a site-specific manner (45–47) to establish the latent infection, which does not lead to obvious consequences for the host cell (47). Thus, this requirement for the lytic cycle can be exploited for vector propagation and/or control of subsequent gene delivery.

Using adenovirus as a helper virus, Kaplitt *et al.* (48) were the first to develop an AAV vector for CNS gene delivery. They generated an AAV vector by replacing the rep and cap genes with lacZ and tyrosine hydroxylase (TH) gene, and demonstrated that administration of AAV vectors resulted in foreign gene expression up to four months in neurons as well as

in glia *in vivo*. In contrast, AAV vectors may also be generated via a “helper-free” system which avoids the helper virus infection. All elements required for AAV production can be provided by transient transfection of helper genes containing plasmids in host cells (49, 50), or by established stable cell lines (51). Recently, self-complementary AAV (scAAV) vectors, sometimes called double-strand AAV, have been developed. The dimeric genomes packaged in scAAV are formed by deleting the terminal resolution site sequence from ITR (52, 53). This results in earlier onset of gene expression and higher transduction efficiency compared with regular single-strand AAV (ssAAV) genomes (54). For gene delivery to the CNS, scAAV vectors have been shown to achieve a better foreign gene transduction than ssAAV (55, 56). However, the insert capacity of scAAV vectors is only half that of ssAAV vectors.

Various cap genes, which determine the AAV serotype, are associated with differential tropism. Several serotypes of AAV have been used for gene delivery to the CNS. Among them, AAV serotype 2 (AAV2) is the predominantly studied and the best characterized serotype in AAV biology. AAV2 exhibits a strong and specific neuronal tropism (57). In addition, AAV2 has a low potential for insertional mutagenesis and appears to be nontoxic when directly injected (58). Most ongoing clinical trials for CNS diseases use AAV2 vectors, and preliminary data suggests that these vectors are well tolerated (59, 60). However, AAV2 has a relatively limited spread through the brain parenchyma (61). Apart from AAV2, several other serotypes have also been tested for gene delivery to the CNS and appear to possess unique traits that can be exploited in different disease contexts. AAV5 transduces both neurons and astrocytes not only at the site of injection but also at more distant sites (62). AAV4-mediated gene delivery is preferential to ependymal cells and astrocytes in SVZ (63). In addition, AAV1, 6, 8 and 9 enable transgene delivery to the neonatal and adult mouse brain with greater efficiency and distribution than AAV2 (64–66). Recently, efficient gene transfer mediated by AAV8 was also observed in monkey brains with a preferential tropism for neurons but not for glia (67). AAV8 and AAV9 have both been studied for gene therapy in various models of CNS disease (68, 69).

Overall, their biological features allow AAV-derived vectors to be promising gene transfer vectors for use in gene therapy. They currently represent the predominant vector for gene therapy in clinical trials for neurodegenerative diseases. Despite a recent study demonstrating that the AAV vector capacity can be expanded from approximately 4.7 to 6 kb by removing the capsid protein subunit Vp2 (70), a significant limitation of AAV vectors is their relatively small cloning capacity compared to other viral vector systems. Thus, the development of alternative larger capacity viral vectors remains a necessity.

### 3.4. Herpes simplex virus

Herpes simplex virus (HSV) contains a double-stranded DNA genome enclosed in a protein capsid surrounded by a lipid membrane envelope. The 152-kb genome of HSV is the largest and most complex of all the viruses being developed for gene therapy. However, vectors derived from HSV, which is a naturally neurotropic human virus, can maintain their presence in sensory neurons for long periods after infection. Thus, they are very attractive and suitable for CNS gene therapy (71–73).

Two types of HSV vector systems have been developed: recombinant virus and amplicons. Recombinant virus can be divided into replication-competent attenuated vectors and replication-defective vectors. Replication-competent attenuated vectors contain essential genes for *in vivo* replication and retain the ability to multiply in actively dividing cells. Replication-defective vectors, on the other hand, are created by deleting immediate early (IE) genes that are required for replication, including the viral thymidine kinase gene. Nonessential viral genes in recombinant HSV vectors can be replaced by transgenes of interest at different sites in the viral genome, providing a large capacity for use in gene therapy. These vectors can usually accommodate up to 40 kb of transgenic DNA. Replication-competent attenuated vectors have been used in Phase I and II human trials for malignant brain tumors (74). However, these vectors exhibit cytopathic effects due to the presence of IE gene products (75). Replication-defective HSV vectors exhibit diminished toxicity, but result in severely reduced transgene expression (76, 77). These vectors are currently being used in a Phase I clinical trial for the treatment of pain (78).

Also known as defective helper-dependent vectors, amplicon vectors contain plasmid DNAs with only minimal HSV replication (*ori*) and packaging (*pac*) DNA sequences in the viral vector genome. Amplicons require HSV helper functions, which usually are provided by recombinant viral vectors. To improve safety, a series of helper-free amplicon vectors has been developed. Today, bacterial artificial chromosome and hybrid amplicons (containing the *ori* and *pac* sequences from other viral vectors) can be used for amplicon packaging, and have been produced in high titers (79). Compared to recombinant HSV vectors, amplicons have reduced cellular toxicity and lower immune response (80). In addition, the capacity to carry large fragments of foreign DNA allows amplicons to be used not only for the insertion of entire genomic loci but also for the addition of various elements, including promoters, inducible systems for regulated gene expression or several separate expression cassettes (81).

HSV vectors have been used in CNS clinical trials for brain tumor therapy (82), but HSV amplicon vectors have not yet been tested in human clinical trials. However, the potential of HSV amplicon vectors for CNS gene therapy is promising based on animal studies (83–87). Overall, the HSV vector system, with its large transgene capacity, offers a useful tool for gene delivery to the CNS that allows for cell-specific and regulated gene expression of multiple genes. Table 1 summarizes key features of different viral vector systems.

### 3.5. Promoter effects on viral vector-mediated transgene expression

The promoter is clearly one of the major elements that influence transgene expression levels. Most viral vector-mediated gene transfer is under the control of strong constitutive promoters. The human cytomegalovirus (CMV) immediate early promoter has been widely used in almost all viral vector systems and is one of the strongest promoters available for gene therapy in most tissues and cells. However, this promoter can be silenced following *in vivo* gene therapy (88), especially in lentivirus-mediated gene delivery. Although it has been reported to drive neuronal gene expression *in vitro* (89), data from our lab (unpublished data) and others (90, 91) demonstrated only weak CMV promoter activity in neurons but strong activity in astrocytes in lentivirus-mediated gene transfer. Other pan-cellular



promoters that have been applied to gene transduction in the CNS include phosphoglycerate kinase 1 (PGK1), elongation factor 1- $\alpha$  (EF1- $\alpha$ ), the viral promoter Rous Sarcoma Virus (RSV), and a hybrid CMV/ $\beta$ -actin (CAG) promoter. All these promoters can successfully drive gene expression in the brain (92–94) but with potential individual differences in efficacy and/or regional preference. For example, the CAG promoter appears to lead to higher transduction efficacy in either striatum or white matter compared to CMV and EF1- $\alpha$  (94). The RSV promoter was shown to exert stronger activity compared to the CMV promoter (90, 92) and produces a region-specific expression in the brain when used with an adenoviral system (92). However, these promoters have not been systematically analyzed in the same vector background. To better understand the efficacy of lentiviral vectors driven by different promoters and the effect of the phenotype of transduced cells in the CNS, we constructed a series of lentiviral vectors driven by CMV, RSV, CAG, EF1- $\alpha$ , ubiquitin C, PGK1, synapsin I and neuron-specific enolase (NSE) promoters. Our results indicate that the synapsin I promoter has the highest transduction efficacy, followed by the constitutive promoter PGK1, in neurons both in primary culture and in the brain. Interestingly, the CMV promoter had the lowest transduction efficacy (unpublished data). As opposed to the differences observed in transduction of neurons, the various viral promoters drove equally high-efficiency gene transduction in glial cells.

The use of cell-specific promoter elements is one approach to achieve targeted transduction in the CNS. NSE, synapsin I, tubulin and platelet-derived growth factor  $\beta$ -chain promoters were commonly chosen for neuron-specific expression. All of these promoters drive persistent and selective neuronal expression *in vitro* and *in vivo* (90, 94–97). However, several lines of evidence (95, 96) and our unpublished data indicate that the synapsin I promoter has the highest specificity for neuronal expression. Our laboratory has tested a wide array of promoters in the context of lentiviral vector gene delivery. We found that synapsin I promoter is the best and strongest promoter in lentivirus-mediated gene delivery for neurons, in the setting of both primary cultures and *in vivo*. As an example of neuronal subtype-specific targeting, tyrosine hydroxylase (TH) upstream promoter can be used as a dopamine neuron-specific promoter. Oh *et al.* (98) demonstrated that TH promoter could specifically drive transgene expression in dopaminergic neurons of the substantia nigra *in vivo*, and may be useful for tracing dopamine neuronal circuits in the brain. In addition, the glial fibrillary acidic protein (GFAP) promoter was demonstrated to selectively drive transgene expression in astrocyte *in vitro* and *in vivo* (94, 99, 100). It was reported that astrocyte-specific expression of GDNF by GFAP promoter could prevent DA neuron degeneration in a Parkinson's disease model (101). Our study also found that viral promoters such as CMV and RSV prefer to transduce astrocytes in lentivirus-mediated gene transfer (unpublished data). Interestingly, the combination of a CMV enhancer with neuronal-specific promoters yielded a higher transgene expression compared to the neuron-specific promoter alone (96, 102). However, for most neuron-specific promoters, the fusion of CMV enhancer significantly decreased their neuronal specificity (96).

Overall, the choice of promoter is crucial for efficiency, specificity and longevity of transgene expression in viral vector-mediated CNS gene delivery. Neuron or glia selective gene delivery can be achieved by using cell-specific promoters, which may be more useful

than constitutive promoters in the context of future gene therapy applications for CNS diseases.

## 4. CEREBROVASCULAR DISEASES

### 4.1. Ischemic stroke

Stroke is the nation's third leading cause of death and the most common cause of permanent disability in adults. About 80% of strokes are ischemic and 15% are hemorrhagic. Thus far, the only drug that has been used successfully to treat acute ischemic stroke in the clinic is the clot-dissolving drug tissue plasminogen activator (tPA). However, tPA must be administered within 3 hours of the onset of an ischemic stroke, making it a viable treatment for less than 5% of patients (103, 104). Thus, strategies providing a wider inclusion of patient populations as well as combinatorial approaches targeting neuroprotective signaling are needed for the treatment of stroke. Delivery of exogenous genes to the brain is believed to be able to alter the post-ischemic pathophysiologic process, and thus has become an attractive treatment for ischemic stroke. Several viral vectors have been used for gene therapy in experimental stroke models. Here we review the application of viral vectors in ischemic stroke based on the type of vector.

**4.1.1. HSV vectors**—HSV vectors containing neuroprotective genes were first tested. Lawrence *et al.* showed that HSV-mediated overexpression of glucose transporter following transient focal cerebral ischemia improved the survival of striatal neurons (105). Since it is well established that apoptotic cell death occurs during cerebral ischemic injury (106), anti-apoptotic genes are another class of candidates for gene therapy against stroke. Bcl-2 is one of the most studied anti-apoptotic genes. Overexpression of this gene by HSV either before (107) or immediately after focal cerebral ischemia (108) was able to prevent stroke-induced loss of neurons in the striatum. In addition to anti-apoptotic proteins, heat shock proteins (HSPs) can also attenuate cerebral ischemic injury. Injection of HSV vectors expressing the inducible form of HSP70 after ischemia significantly improved striatal neuron survival (109). However, HSP70 or Bcl-2 overexpression by HSV vectors were no longer protective when delivered 5 hours after ischemia (108, 109). Thus, other genes and vector systems are still under investigation in order to further expand the post-ischemic therapy window.

**4.1.2. AAV vectors**—AAV vectors have become the most commonly used vehicles for ischemic stroke gene therapy. AAV vector-mediated Bcl-2 overexpression prevented ischemia-induced DNA fragment in the CA1 region of gerbil hippocampus (110). Similarly, AAV-mediated Bcl-w overexpression in cerebral cortex and striatum significantly reduced infarct volume and improved neurological function (111). In addition, our laboratory (112) identified a novel anti-apoptosis protein designated as Apaf-1-interacting protein (AIP), which functions as an inhibitor of the Apaf-1-caspase-9 pathway. AAV-mediated overexpression of AIP in the hippocampus significantly promoted neuronal survival after transient global cerebral ischemia.

Neurotrophins are another class of candidate genes for stroke gene therapy. These genes include brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF) and nerve growth factor (NGF), all of which have substantial evidence supporting a



role in neuroprotection and attenuation of ischemic neuronal injury. AAV-mediated delivery of BDNF or NGF to the striatum 4–5 weeks prior to ischemia moderately mitigated neuronal death following stroke and led to detectable functional sparing in a rat transient MCAO model (113). Similarly, AAV-based GDNF gene delivery immediately after ischemia was reported to rescue cortical neuronal cells by preventing apoptosis (114). GDNF delivery by adenovirus or HSV was also shown to decrease apoptotic cells and protect against ischemic injury in various ischemic animal models (84, 115, 116). However, long-term AAV-mediated high levels of GDNF were also shown to provide no protection to striatal neurons after MCAO; in fact, they exacerbated ischemia-induced neuronal loss and, moreover, failed to promote functional recovery (117). In addition, AAV-mediated BDNF delivery to hippocampus was shown to suppress ischemia-induced neurogenesis in a rat stroke model (118). However, conflicting results were observed by Gustafsson *et al.*, who found that high levels of BDNF release via AAV retrograde axonal transport increased the vulnerability of striatal interneurons to stroke-induced damage (119). The above results indicate that viral vector-based neurotrophin gene therapy for stroke may be more complex than previously assumed. More research is needed to further assess its effects on stroke.

In addition to rescuing ischemia-induced neuronal loss, viral vector-based gene therapy for stroke also can aim to promote post-ischemic angiogenesis and neurogenesis. It is established that vascular endothelial growth factor (VEGF) plays multiple roles in post-ischemic pathophysiologic progress. In a gerbil global cerebral ischemic model, injection of AAV-VEGF into the lateral ventricle was reported to significantly attenuate CA1 delayed neuronal death and post-ischemic learning deficits (120). However, systemic delivery of VEGF increased BBB damage and induced brain edema in the early phase after ischemia (121). To reduce VEGF-induced BBB disruption in cerebral ischemia, an AAV vector containing VEGF under the control of hypoxia-responsive element was constructed (122). Injection of this VEGF vector reduced the infarct volume and neuronal loss induced by transient MCAO. Inclusion of the hypoxia-responsive element also restricts VEGF expression to areas of ischemia and minimizes its side-effects. Another approach to reduce VEGF-induced BBB damage is the combined use of AAV encoding VEGF and Angiopoietin 1 (Ang1), which can reduce permeability of endothelial cells induced by VEGF (123). Co-transduction of AAV-Ang1 enhanced VEGF-mediated formation of mature neovessels and protection against ischemic injury, with reduced side-effects on BBB permeability. Apart from VEGF, insulin-like growth factor-1 (IGF-1) was recently used for stroke gene therapy. Overexpression of IGF-1 prior to ischemic injury through an AAV transduction system promoted long-term functional recovery due to enhanced neovascularization and neurogenesis (124). A similar effect was also achieved by injection of IGF-1 expressing AAV vector at 24 hours after stroke (125), suggesting AAV-based IGF-1 gene delivery may provide a long therapeutic time window.

**4.1.3. Lentiviral vectors**—Lentiviral vectors also provide potential therapeutic tools for use in cerebral ischemic stroke. Human anti-apoptotic Bcl-2 or GDNF genes overexpressed by lentiviral vectors protected against excitotoxic damage during ischemia in both cultured hippocampal neurons and rat hippocampus (9). Lentiviral vector-delivered matrix metalloproteinase (MMP-9) shRNA also ameliorated ischemic brain injury in rats by

increasing BBB integrity (126). Besides direct injection, lentiviral vectors can also be delivered to ischemic brains via neural stem cell transplantation. Transduced neural stem cells survive and secrete neurotrophic factors, such as NT-3, and enhance functional recovery after stroke in rats (19). In addition, Daadi and colleagues combined bioluminescence and magnetic resonance imaging (MRI) to show that neural stem cells survived for 2 months after transplantation and differentiated into neurons, oligodendrocytes and astrocytes in stroke-damaged rat brains (127). Real-time imaging provides an important non-invasive tool useful for tracking and monitoring neural stem cell fate and function in ischemic brain environments, and this is very important for successful delivery and safety in the clinical setting.

Accumulating evidence indicates that viral vector-mediated gene therapy may be a therapeutically useful strategy for stroke therapy. Exogenous gene expression alters post-ischemic pathophysiologic progress and improves recovery from ischemic injury. However, the ability to use viral vector-mediated gene transfer for treating stroke will be dependent upon the optimization of the selection of targeted gene(s) as well as the development of non-neurosurgical approaches to administer viral vectors. Further studies focusing on the delivery method of viral vector will be helpful to determine the viability of virus-based gene therapy for human stroke.

**4.1.4. RNA interference**—RNA interference (RNAi) has been developed as a promising tool to specifically downregulate candidate genes. RNAi-mediated gene silencing can be initiated by synthetic double-stranded small interfering RNA (siRNA) or by short hairpin RNA (shRNA), which can be processed to siRNA inside cells. Viral vectors are ideal vehicles for shRNA delivery. By using proper viral vectors, it is possible to achieve persistent shRNA expression and targeted gene knockdown *in vivo*. For the CNS, lentiviral and AAV vectors have been used in this capacity and are able to produce long-term genetic knockdown (128–131). The most commonly used promoters for shRNA focus on ubiquitously expressed polymerase III promoters, such as U6 and H1. Both of these promoters have been successfully utilized to drive viral vector-mediated shRNA expression in the brain (126, 132). Recently, lentiviral vectors expressing shRNA under the control of a polymerase II promoter from CMV knocked down gene expression in the brain (129). In particular, this study also showed neuron-specific gene knockdown using the NSE promoter, offering the possibility of specifically targeting RNAi in the brain (129). In the context of cerebral ischemia, recent studies demonstrated the therapeutic potential of viral vector-based RNAi. Since the calpain family of cysteine proteases plays a causal role in neuronal cell death following acute brain injury (133, 134), Bevers *et al.* (135) constructed AAV vectors expressing shRNAs targeting micro- and m-calpain. They subsequently demonstrated that knockdown of micro-calpain could decrease neuronal death and preserve hippocampal function in a global cerebral ischemic model (135).

**4.1.5. Global cerebral ischemia**—Global cerebral ischemia occurs when blood flow to most or all of the brain is halted or drastically reduced, commonly caused by cardiac arrest. Global brain ischemia leads to delayed neuronal death and cognitive impairment. The symptoms may be transient or permanent dependent on whether sufficient circulation is

restored within a short period of time. Compared to studies using focal ischemic models, significantly fewer viral therapy studies have been investigated in the context of global cerebral ischemia. With the exception of one study utilizing HSV-amplicon to express human Bcl-2 in the CA1 region of the gerbil hippocampus to protect CA1 pyramidal cells from delayed neuronal death (136), and another study using a Sendai viral vector (developed by Hasegawa's group) to mediate neurotrophic factor expression in yielding neuroprotection in a transient global cerebral ischemia model (137, 138), most viral therapies in global cerebral ischemia have been conducted using adenoviral and AAV vectors. Adenovirus-mediated expression of GDNF and tissue inhibitors of matrix metalloproteinases have been shown to significantly prevent the loss of hippocampal CA1 pyramidal neurons following global cerebral ischemia (116, 139). Moreover, Matsuoka and colleagues demonstrated that administration of adenovirus expressing fibroblast growth factor 2 (FGF-2) promoted progenitor cell proliferation more efficiently compared to continuous intraventricular infusion of FGF-2 recombinant protein following transient global ischemia (140). AAV vectors are also widely used for gene expression or suppression in global cerebral ischemia animal models by injection into the hippocampal CA1 region in most cases. With the use of such AAV-mediated gene regulation, roles for the transient receptor potential melastatin 7 (141), Apaf-1 interacting protein (112), and calpain (134, 135) have been explored in delayed neuronal death following global cerebral ischemia. Interestingly, use of viral vectors can alter the effects of a target protein when compared to recombinant protein delivery. For example, Larsson and colleagues showed that long-term expression of BDNF via viral vector in hippocampus inhibited the formation of new dentate granule cells triggered by global cerebral ischemia in rats (118), contrasting with many previous reports demonstrating that recombinant BDNF administration in the lateral ventricle or ventricular zone stimulates neurogenesis. These apparently conflicting reports warn us that long-term expression of neuroprotective molecules by viral vector may exert differential effects compared to protein delivery, and thus must be tested carefully before clinical application.

#### 4.2. Hemorrhagic stroke

While ischemic stroke is caused by interruption of the blood supply, hemorrhagic stroke results from the rupture of a blood vessel or an abnormal vascular structure. Although few studies focus on gene therapy for hemorrhagic stroke, emerging findings have revealed alterations in gene expression and protein after hemorrhagic stroke such as intracerebral hemorrhage (ICH) (142), and indicate that gene-targeted strategies may be an attractive treatment to promote recovery from hemorrhagic stroke.

ICH, caused by bleeding within the brain tissue, is one of the main types of hemorrhagic stroke. A hematoma in the brain may produce edema through chemical toxicity and/or mass effect. Furthermore, ICH can result in the disruption of the BBB surrounding the hematoma, and lead to cerebral edema. These events are coincident with inflammation, characterized by production of pro-inflammatory cytokines, activation of resident brain microglia and migration of peripheral immune cells into the brain. Upregulation of inflammatory genes, such as IL-1 $\beta$ , is associated with BBB damage. Adenovirus can be used as a vector to transfer the IL-1 receptor antagonist (IL-1ra), a cytokine competitively inhibiting the binding of IL-1 to its receptor, to the brain subject to experimental ICH. Adenovirus-mediated

overexpression of IL-1ra attenuated brain edema formation and thrombin-induced intracerebral inflammation (polymorphonuclear leukocyte infiltration) following ICH (143). In addition, studies have provided several attractive candidates for ICH gene therapy. Peroxisome proliferator-activated receptor gamma (PPAR- $\gamma$ ) may be one of these candidate genes because it acts as an important gene in promoting phagocytosis of microglia and hematoma absorption, resulting in protection of surrounding brain cells from ICH-induced damage (144). PPAR- $\gamma$  activator also increases PPAR-regulated gene expression (*e.g.*, catalase and CD36), thus reducing pro-inflammatory gene expression and extracellular H<sub>2</sub>O<sub>2</sub> level (144). Heat-shock proteins (HSPs) may also be good candidates for use against ICH damage, due to their ability to reduce inflammation and apoptosis in a variety of brain insults. Supporting this idea, pharmacological induction of HSP70 was indicated to reduce brain edema and exert neuroprotective effects in experimental ICH (145).

Viral vector-mediated gene transfer has been applied to *ex vivo* gene therapy for ICH. By using a retroviral vector encoding v-myc, Lee *et al.* generated stable, immortalized cell lines of human neural stem cells from primary human fetal telencephalon cultures. Transplantation of these NSCs promoted functional recovery in a mouse ICH model (146). Furthermore, retroviral vectors were used to transfer various genes, including VEGF (147), GDNF (148), BDNF (149), and Akt (150), to this NSC line. These genetically modified NSCs increased survival and differentiation of grafted NSCs, and improved behavioral recovery in a mouse ICH model as compared to parental NSCs.

### 4.3. Subarachnoid hemorrhage

SAH is another main type of hemorrhagic stroke, resulting from blood extravasations into the subarachnoid space. Vasospasm is an important complication of SAH. Adenovirus has been shown to be an attractive vehicle for vasospasm-targeted SAH gene therapy. Intracisternal injection of adenoviral vectors containing  $\beta$ -gal under the control of the CMV promoter successfully expressed the reporter gene in adventitia of blood vessels after SAH (151), suggesting recombinant viral vectors can be used for gene transfer to cerebral blood vessels even in the presence of cisternal blood, and may be useful for preventing cerebral vasospasm after SAH. One of the possible candidate genes for vasospasm-targeted gene therapy is nitric oxide synthase (NOS) because the attenuation of vasospastic response could result from enhanced production of nitric oxide via activation of NOS. Likewise, expression of heme oxygenase-1 or constitutive heme oxygenase-2 can lead to the synthesis of carbon monoxide and similarly attenuate the vasospastic response in rats subjected to experimental SAH (152). However, iNOS knockout mice were shown to have significantly less brain edema than their littermates after ICH when hematoma size was similar, suggesting the induction of iNOS may promote edema formation (153). Therefore, careful investigation concerning the pathological contexts must be performed prior to applying these genes in hemorrhage therapy.

## 5. NON-VIRAL GENE DELIVERY COMPARED TO VIRAL VECTOR

Although viral delivery has dominated the gene therapy field, non-viral gene delivery has made significant strides recently and, if efficiency improves, may yield significant

advantages over traditional viral vectors, such as convenient delivery and reduced risk of pathogenic and immunological complications (154). Non-viral vectors include naked DNA itself or chemical carrier-mediated transgenic DNA ferried to the cellular target (155). Owing to rapid clearance by serum nucleases and the mononuclear phagocyte system, the application of naked DNA delivery is limited. Thus, several kinds of chemical carriers have been designed to improve gene expression and delivery efficiency. Lipids and polymers are the primary carriers for non-viral gene delivery. Recent progress in non-viral vector gene delivery *in vivo* has been made in the use of nanoparticles. Several nanoparticles have been demonstrated to transfer report gene to the brain efficiently. For efficient brain-targeting gene delivery, biodegradable polyamidoamine (PAMAM) esters are being explored and developed (156). Kim *et al.* (157) showed that the transfection of HMGB1 siRNA with PAMAM ester markedly reduced infarct volume in the postischemic brain. Modification of the PAMAM to transferrin (Tf)-conjugated, pegylated PAMAME gives the advantage of accessing the brain from blood via transferrin-receptor-mediated transcytosis and delivering exogenous genes into the brain without damaging the BBB or requiring intracerebroventricular injections. Widespread expression of an exogenous gene was observed in mouse brain after i.v. administration of Tf-conjugated pegylated nanoparticle. However, non-viral vectors still have significant disadvantages when compared to viral vector gene therapy. Typically, viral vectors lead to a higher level of transfection and subsequent expression of inserted genes. Viral-mediated gene transfer may overcome the obstacles to CNS gene delivery due to a natural ability to cross cellular membranes efficiently and to infect post-mitotic cells. The ability of some viral vectors to integrate into the host genome and lead to stable long-term expression render them quite suitable for the treatment of CNS diseases, especially chronic neurological disorders. Furthermore, higher transduction efficacy and long-term stable expression of some classes of viral vectors, especially lentivirus, AAV, and HSV, make them attractive carrier systems for introducing genes into the brain, both for purposes of neurobiological research and for gene therapy of neurological diseases (158).

In addition to direct application of genes to the brain, genes can be transferred to target tissue using genetically modified cells which have been transduced with viral vectors encoding therapeutic genes. Although non-neural cell types were first used for *ex vivo* CNS gene delivery (159), the strong tropism of neural stem cells (NSCs) for the nervous system (160) make them an ideal vehicle for this approach. NSC-mediated gene delivery as a therapeutic tool has been tested in various neurological disease models. As discussed above, transplantation of retroviral vector immortalized human NSCs promoted functional recovery in a mouse ICH model (146). Administration of gene-modified NSCs was able to not only prevent neuronal death but also improve neurological recovery following focal cerebral ischemia (146–150, 161–163). Although NSC-mediated gene delivery combining gene and cell therapy has great potential for the treatment of CNS disease, there are still many hurdles that need to be overcome for its use in therapy.

## 5. SUMMARY

In recent decades, gene therapy has become a highly feasible clinical option for cerebrovascular diseases. This field will continue benefiting from improvements in

elucidating mechanisms of molecular neuropathology and optimizing sophisticated technologies, including RNA interference and optogenetics. Although the CNS faces specific obstacles in terms of gene delivery, various viral vectors have been successfully utilized to deliver genes to the CNS, indicating viral vectors as ideal vehicles for CNS gene therapy. However, the delivery and diffusion of viruses throughout large brain structures and the sustained expression of gene candidates via viral vectors still need to be improved significantly. Further studies on the safety and toxicity of viral vectors, and potential inflammatory and immune responses to them, are required. Carefully addressing these hurdles may move gene therapeutic approaches for cerebrovascular diseases from pre-clinical neuroscience to a clinical reality.

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## References

1. Snyder BR, Boulis NM, Federici T. Viral vector-mediated gene transfer for CNS disease. *Expert Opin Biol Ther.* 2010; 10(3):381–94. [PubMed: 20132059]
2. Tang G, Chiocca A. Gene transfer and delivery in central nervous system disease. *Neurosurg Focus.* 1997; 3(3):e2.
3. Azzouz M, Kingsman SM, Mazarakis ND. Lentiviral vectors for treating and modeling human CNS disorders. *J Gene Med.* 2004; 6(9):951–62. [PubMed: 15352068]
4. Lim ST, Airavaara M, Harvey BK. Viral vectors for neurotrophic factor delivery: a gene therapy approach for neurodegenerative diseases of the CNS. *Pharmacol Res.* 2010; 61(1):14–26. [PubMed: 19840853]
5. Naldini L, Blomer U, Gallay P, Ory D, Mulligan R, Gage FH, Verma IM, Trono D. In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. *Science.* 1996; 272(5259):263–7. [PubMed: 8602510]
6. Zufferey R, Nagy D, Mandel RJ, Naldini L, Trono D. Multiply attenuated lentiviral vector achieves efficient gene delivery in vivo. *Nat Biotechnol.* 1997; 15(9):871–5. [PubMed: 9306402]
7. Dull T, Zufferey R, Kelly M, Mandel RJ, Nguyen M, Trono D, Naldini L. A third-generation lentivirus vector with a conditional packaging system. *J Virol.* 1998; 72(11):8463–71. [PubMed: 9765382]
8. Zufferey R, Dull T, Mandel RJ, Bukovsky A, Quiroz D, Naldini L, Trono D. Self-inactivating lentivirus vector for safe and efficient in vivo gene delivery. *J Virol.* 1998; 72(12):9873–80. [PubMed: 9811723]
9. Wong LF, Ralph GS, Walmsley LE, Bienemann AS, Parham S, Kingsman SM, Uney JB, Mazarakis ND. Lentiviral-mediated delivery of Bcl-2 or GDNF protects against excitotoxicity in the rat hippocampus. *Mol Ther.* 2005; 11(1):89–95. [PubMed: 15585409]
10. Bjorklund A, Kirik D, Rosenblad C, Georgievska B, Lundberg C, Mandel RJ. Towards a neuroprotective gene therapy for Parkinson's disease: use of adenovirus, AAV and lentivirus vectors for gene transfer of GDNF to the nigrostriatal system in the rat Parkinson model. *Brain Res.* 2000; 886(1–2):82–98. [PubMed: 11119690]
11. de Almeida LP, Zala D, Aebischer P, Deglon N. Neuroprotective effect of a CNTF-expressing lentiviral vector in the quinolinic acid rat model of Huntington's disease. *Neurobiol Dis.* 2001; 8(3):433–46. [PubMed: 11442352]
12. Bemelmans AP, Husson I, Jaquet M, Mallet J, Kosofsky BE, Gressens P. Lentiviral-mediated gene transfer of brain-derived neurotrophic factor is neuroprotective in a mouse model of neonatal excitotoxic challenge. *J Neurosci Res.* 2006; 83(1):50–60. [PubMed: 16299771]



13. Slaets H, Hendriks JJ, Van den Haute C, Coun F, Baekelandt V, Stinissen P, Hellings N. CNS-targeted LIF expression improves therapeutic efficacy and limits autoimmune-mediated demyelination in a model of multiple sclerosis. *Mol Ther.* 2010; 18(4):684–91. [PubMed: 20068552]
14. Azzouz M, Ralph S, Wong LF, Day D, Askham Z, Barber RD, Mitrophanous KA, Kingsman SM, Mazarakis ND. Neuroprotection in a rat Parkinson model by GDNF gene therapy using EIAV vector. *Neuroreport.* 2004; 15(6):985–90. [PubMed: 15076720]
15. Dowd E, Monville C, Torres EM, Wong LF, Azzouz M, Mazarakis ND, Dunnett SB. Lentivector-mediated delivery of GDNF protects complex motor functions relevant to human Parkinsonism in a rat lesion model. *Eur J Neurosci.* 2005; 22(10):2587–95. [PubMed: 16307601]
16. Valori CF, Ning K, Wyles M, Azzouz M. Development and applications of non-HIV-based lentiviral vectors in neurological disorders. *Curr Gene Ther.* 2008; 8(6):406–18. [PubMed: 19075624]
17. Burns JC, Friedmann T, Driever W, Burrascano M, Yee JK. Vesicular stomatitis virus G glycoprotein pseudotyped retroviral vectors: concentration to very high titer and efficient gene transfer into mammalian and nonmammalian cells. *Proc Natl Acad Sci U S A.* 1993; 90(17):8033–7. [PubMed: 8396259]
18. Naldini L, Blomer U, Gage FH, Trono D, Verma IM. Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a lentiviral vector. *Proc Natl Acad Sci U S A.* 1996; 93(21):11382–8. [PubMed: 8876144]
19. Zhang ZH, Wang RZ, Li GL, Wei JJ, Li ZJ, Feng M, Kang J, Du WC, Ma WB, Li YN, Yang Y, Kong YG. Transplantation of neural stem cells modified by human neurotrophin-3 promotes functional recovery after transient focal cerebral ischemia in rats. *Neurosci Lett.* 2008; 444(3): 227–30. [PubMed: 18760326]
20. Charlton KM. The pathogenesis of rabies and other lyssaviral infections: recent studies. *Curr Top Microbiol Immunol.* 1994; 187:95–119. [PubMed: 7859501]
21. Watson DJ, Kobinger GP, Passini MA, Wilson JM, Wolfe JH. Targeted transduction patterns in the mouse brain by lentivirus vectors pseudotyped with VSV, Ebola, Mokola, LCMV, or MuLV envelope proteins. *Mol Ther.* 2002; 5(5 Pt 1):528–37. [PubMed: 11991743]
22. Wong LF, Azzouz M, Walmsley LE, Askham Z, Wilkes FJ, Mitrophanous KA, Kingsman SM, Mazarakis ND. Transduction patterns of pseudotyped lentiviral vectors in the nervous system. *Mol Ther.* 2004; 9(1):101–11. [PubMed: 14741783]
23. Mazarakis ND, Azzouz M, Rohll JB, Ellard FM, Wilkes FJ, Olsen AL, Carter EE, Barber RD, Baban DF, Kingsman SM, Kingsman AJ, O'Malley K, Mitrophanous KA. Rabies virus glycoprotein pseudotyping of lentiviral vectors enables retrograde axonal transport and access to the nervous system after peripheral delivery. *Hum Mol Genet.* 2001; 10(19):2109–21. [PubMed: 11590128]
24. Mentis GZ, Gravell M, Hamilton R, Shneider NA, O'Donovan MJ, Schubert M. Transduction of motor neurons and muscle fibers by intramuscular injection of HIV-1-based vectors pseudotyped with select rabies virus glycoproteins. *J Neurosci Methods.* 2006; 157(2):208–17. [PubMed: 16725205]
25. Azzouz M, Le T, Ralph GS, Walmsley L, Monani UR, Lee DC, Wilkes F, Mitrophanous KA, Kingsman SM, Burghes AH, Mazarakis ND. Lentivector-mediated SMN replacement in a mouse model of spinal muscular atrophy. *J Clin Invest.* 2004; 114(12):1726–31. [PubMed: 15599397]
26. Consiglio A, Gritti A, Dolcetta D, Follenzi A, Bordignon C, Gage FH, Vescovi AL, Naldini L. Robust in vivo gene transfer into adult mammalian neural stem cells by lentiviral vectors. *Proc Natl Acad Sci U S A.* 2004; 101(41):14835–40. [PubMed: 15466696]
27. Yamane J, Nakamura M, Iwanami A, Sakaguchi M, Katoh H, Yamada M, Momoshima S, Miyao S, Ishii K, Tamaoki N, Nomura T, Okano HJ, Kanemura Y, Toyama Y, Okano H. Transplantation of galectin-1-expressing human neural stem cells into the injured spinal cord of adult common marmosets. *J Neurosci Res.* 2010; 88(7):1394–405. [PubMed: 20091712]
28. Ebert AD, Barber AE, Heins BM, Svendsen CN. Ex vivo delivery of GDNF maintains motor function and prevents neuronal loss in a transgenic mouse model of Huntington's disease. *Exp Neurol.* 2010; 224(1):155–62. [PubMed: 20227407]

29. Sarkis C, Philippe S, Mallet J, Serguera C. Non-integrating lentiviral vectors. *Curr Gene Ther.* 2008; 8(6):430–7. [PubMed: 19075626]
30. Rahim AA, Wong AM, Howe SJ, Buckley SM, Acosta-Saltos AD, Elston KE, Ward NJ, Philpott NJ, Cooper JD, Anderson PN, Waddington SN, Thrasher AJ, Raivich G. Efficient gene delivery to the adult and fetal CNS using pseudotyped non-integrating lentiviral vectors. *Gene Ther.* 2009; 16(4):509–20. [PubMed: 19158847]
31. Breyer B, Jiang W, Cheng H, Zhou L, Paul R, Feng T, He TC. Adenoviral vector-mediated gene transfer for human gene therapy. *Curr Gene Ther.* 2001; 1(2):149–62. [PubMed: 12108952]
32. Shirakawa T. Clinical trial design for adenoviral gene therapy products. *Drug News Perspect.* 2009; 22(3):140–5. [PubMed: 19440556]
33. Wood MJ, Charlton HM, Wood KJ, Kajiwara K, Byrnes AP. Immune responses to adenovirus vectors in the nervous system. *Trends Neurosci.* 1996; 19(11):497–501. [PubMed: 8931276]
34. Byrnes AP, MacLaren RE, Charlton HM. Immunological instability of persistent adenovirus vectors in the brain: peripheral exposure to vector leads to renewed inflammation, reduced gene expression, and demyelination. *J Neurosci.* 1996; 16(9):3045–55. [PubMed: 8622134]
35. Thomas CE, Schiedner G, Kochanek S, Castro MG, Lowenstein PR. Peripheral infection with adenovirus causes unexpected long-term brain inflammation in animals injected intracranially with first-generation, but not with high-capacity, adenovirus vectors: toward realistic long-term neurological gene therapy for chronic diseases. *Proc Natl Acad Sci U S A.* 2000; 97(13):7482–7. [PubMed: 10840055]
36. Amalfitano A, Hauser MA, Hu H, Serra D, Begy CR, Chamberlain JS. Production and characterization of improved adenovirus vectors with the E1, E2b, and E3 genes deleted. *J Virol.* 1998; 72(2):926–33. [PubMed: 9444984]
37. Lusky M, Christ M, Rittner K, Dieterle A, Dreyer D, Mourot B, Schultz H, Stoeckel F, Pavirani A, Mehtali M. In vitro and in vivo biology of recombinant adenovirus vectors with E1, E1/E2A, or E1/E4 deleted. *J Virol.* 1998; 72(3):2022–32. [PubMed: 9499056]
38. Alba R, Bosch A, Chillon M. Gutless adenovirus: last-generation adenovirus for gene therapy. *Gene Ther.* 2005; 12(Suppl 1):S18–27. [PubMed: 16231052]
39. Russell WC. Update on adenovirus and its vectors. *J Gen Virol.* 2000; 81(Pt 11):2573–604. [PubMed: 11038369]
40. Barcia C, Jimenez-Dalmaroni M, Kroeger KM, Puntel M, Rapaport AJ, Larocque D, King GD, Johnson SA, Liu C, Xiong W, Candolfi M, Mondkar S, Ng P, Palmer D, Castro MG, Lowenstein PR. One-year expression from high-capacity adenoviral vectors in the brains of animals with pre-existing anti-adenoviral immunity: clinical implications. *Mol Ther.* 2007; 15(12):2154–63. [PubMed: 17895861]
41. Soudais C, Skander N, Kremer EJ. Long-term in vivo transduction of neurons throughout the rat CNS using novel helper-dependent CAV-2 vectors. *FASEB J.* 2004; 18(2):391–3. [PubMed: 14688208]
42. Thomas CE, Birkett D, Anozie I, Castro MG, Lowenstein PR. Acute direct adenoviral vector cytotoxicity and chronic, but not acute, inflammatory responses correlate with decreased vector-mediated transgene expression in the brain. *Mol Ther.* 2001; 3(1):36–46. [PubMed: 11162309]
43. Snyder RO, Flotte TR. Production of clinical-grade recombinant adeno-associated virus vectors. *Curr Opin Biotechnol.* 2002; 13(5):418–23. [PubMed: 12459331]
44. Cao L, Doring M, Xiao W. Replication competent helper functions for recombinant AAV vector generation. *Gene Ther.* 2002; 9(18):199–206. [PubMed: 12215886]
45. Samulski RJ, Zhu X, Xiao X, Brook JD, Housman DE, Epstein N, Hunter LA. Targeted integration of adeno-associated virus (AAV) into human chromosome 19. *EMBO J.* 1991; 10(12):3941–50. [PubMed: 1657596]
46. Shelling AN, Smith MG. Targeted integration of transfected and infected adeno-associated virus vectors containing the neomycin resistance gene. *Gene Ther.* 1994; 1(3):165–9. [PubMed: 7584077]
47. Kotin RM, Siniscalco M, Samulski RJ, Zhu XD, Hunter L, Laughlin CA, McLaughlin S, Muzyczka N, Rocchi M, Berns KI. Site-specific integration by adeno-associated virus. *Proc Natl Acad Sci U S A.* 1990; 87(6):2211–5. [PubMed: 2156265]

48. Kaplitt MG, Leone P, Samulski RJ, Xiao X, Pfaff DW, O'Malley KL, During MJ. Long-term gene expression and phenotypic correction using adeno-associated virus vectors in the mammalian brain. *Nat Genet.* 1994; 8(2):148–54. [PubMed: 7842013]
49. Grimm D, Kleinschmidt JA. Progress in adeno-associated virus type 2 vector production: promises and prospects for clinical use. *Hum Gene Ther.* 1999; 10(15):2445–50. [PubMed: 10543610]
50. Xiao X, Li J, Samulski RJ. Production of high-titer recombinant adeno-associated virus vectors in the absence of helper adenovirus. *J Virol.* 1998; 72(3):2224–32. [PubMed: 9499080]
51. Qiao C, Wang B, Zhu X, Li J, Xiao X. A novel gene expression control system and its use in stable, high-titer 293 cell-based adeno-associated virus packaging cell lines. *J Virol.* 2002; 76(24):13015–27. [PubMed: 12438627]
52. McCarty DM, Fu H, Monahan PE, Toulson CE, Naik P, Samulski RJ. Adeno-associated virus terminal repeat (TR) mutant generates self-complementary vectors to overcome the rate-limiting step to transduction in vivo. *Gene Ther.* 2003; 10(26):2112–8. [PubMed: 14625565]
53. Wang Z, Ma HI, Li J, Sun L, Zhang J, Xiao X. Rapid and highly efficient transduction by double-stranded adeno-associated virus vectors in vitro and in vivo. *Gene Ther.* 2003; 10(26):2105–11. [PubMed: 14625564]
54. McCarty DM. Self-complementary AAV vectors; advances and applications. *Mol Ther.* 2008; 16(10):1648–56. [PubMed: 18682697]
55. Duque S, Joussemet B, Riviere C, Marais T, Dubreil L, Douar AM, Fyfe J, Moullier P, Colle MA, Barkats M. Intravenous administration of self-complementary AAV9 enables transgene delivery to adult motor neurons. *Mol Ther.* 2009; 17(7):1187–96. [PubMed: 19367261]
56. McCarty DM, DiRosario J, Gulaid K, Muenzer J, Fu H. Mannitol-facilitated CNS entry of rAAV2 vector significantly delayed the neurological disease progression in MPS IIIB mice. *Gene Ther.* 2009; 16(11):1340–52. [PubMed: 19587708]
57. Taymans JM, Vandenberghe LH, Haute CV, Thiry I, Deroose CM, Mortelmans L, Wilson JM, Debysier Z, Baekelandt V. Comparative analysis of adeno-associated viral vector serotypes 1, 2, 5, 7, and 8 in mouse brain. *Hum Gene Ther.* 2007; 18(3):195–206. [PubMed: 17343566]
58. McCown TJ. Adeno-associated virus (AAV) vectors in the CNS. *Curr Gene Ther.* 2005; 5(3):333–8. [PubMed: 15975010]
59. Bartus RT, Herzog CD, Bishop K, Ostrove JM, Tuszynski M, Kordower JH, Gasmi M. Issues regarding gene therapy products for Parkinson's disease: the development of CERE-120 (AAV-NTN) as one reference point. *Parkinsonism Relat Disord.* 2007; 13(Suppl 3):S469–77. [PubMed: 18267286]
60. Marks WJ Jr, Ostrem JL, Verhagen L, Starr PA, Larson PS, Bakay RA, Taylor R, Cahn-Weiner DA, Stoessl AJ, Olanow CW, Bartus RT. Safety and tolerability of intraputaminally delivered CERE-120 (adeno-associated virus serotype 2-neurturin) to patients with idiopathic Parkinson's disease: an open-label, phase I trial. *Lancet Neurol.* 2008; 7(5):400–8. [PubMed: 18387850]
61. McFarland NR, Lee JS, Hyman BT, McLean PJ. Comparison of transduction efficiency of recombinant AAV serotypes 1, 2, 5, and 8 in the rat nigrostriatal system. *J Neurochem.* 2009; 109(3):838–45. [PubMed: 19250335]
62. Davidson BL, Stein CS, Heth JA, Martins I, Kotin RM, Derksen TA, Zabner J, Ghodsi A, Chiorini JA. Recombinant adeno-associated virus type 2, 4, and 5 vectors: transduction of variant cell types and regions in the mammalian central nervous system. *Proc Natl Acad Sci U S A.* 2000; 97(7):3428–32. [PubMed: 10688913]
63. Liu G, Martins IH, Chiorini JA, Davidson BL. Adeno-associated virus type 4 (AAV4) targets ependyma and astrocytes in the subventricular zone and RMS. *Gene Ther.* 2005; 12(20):1503–8. [PubMed: 15944733]
64. Huszthy PC, Svendsen A, Wilson JM, Kotin RM, Lonning PE, Bjerkvig R, Hoover F. Widespread dispersion of adeno-associated virus serotype 1 and adeno-associated virus serotype 6 vectors in the rat central nervous system and in human glioblastoma multiforme xenografts. *Hum Gene Ther.* 2005; 16(3):381–92. [PubMed: 15812233]
65. Foust KD, Nurre E, Montgomery CL, Hernandez A, Chan CM, Kaspar BK. Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. *Nat Biotechnol.* 2009; 27(1):59–65. [PubMed: 19098898]

66. Broekman ML, Comer LA, Hyman BT, Sena-Esteves M. Adeno-associated virus vectors serotyped with AAV8 capsid are more efficient than AAV-1 or -2 serotypes for widespread gene delivery to the neonatal mouse brain. *Neuroscience*. 2006; 138(2):501–10. [PubMed: 16414198]
67. Masamizu Y, Okada T, Ishibashi H, Takeda S, Yuasa S, Nakahara K. Efficient gene transfer into neurons in monkey brain by adeno-associated virus 8. *Neuroreport*. 2010; 21(6):447–51. [PubMed: 20224455]
68. Passini MA, Bu J, Roskelley EM, Richards AM, Sardi SP, O’Riordan CR, Klingler KW, Shihabuddin LS, Cheng SH. CNS-targeted gene therapy improves survival and motor function in a mouse model of spinal muscular atrophy. *J Clin Invest*. 2010; 120(4):1253–64. [PubMed: 20234094]
69. Xue YQ, Ma BF, Zhao LR, Tatom JB, Li B, Jiang LX, Klein RL, Duan WM. AAV9-mediated erythropoietin gene delivery into the brain protects nigral dopaminergic neurons in a rat model of Parkinson’s disease. *Gene Ther*. 2010; 17(1):83–94. [PubMed: 19727138]
70. Grieger JC, Samulski RJ. Packaging capacity of adeno-associated virus serotypes: impact of larger genomes on infectivity and postentry steps. *J Virol*. 2005; 79(15):9933–44. [PubMed: 16014954]
71. Berges BK, Wolfe JH, Fraser NW. Transduction of brain by herpes simplex virus vectors. *Mol Ther*. 2007; 15(1):20–9. [PubMed: 17164771]
72. Kennedy PG. Potential use of herpes simplex virus (HSV) vectors for gene therapy of neurological disorders. *Brain*. 1997; 120(Pt 7):1245–59. [PubMed: 9236634]
73. Goss JR, Gold MS, Glorioso JC. HSV vector-mediated modification of primary nociceptor afferents: an approach to inhibit chronic pain. *Gene Ther*. 2009; 16(4):493–501. [PubMed: 19357694]
74. Harrow S, Papanastassiou V, Harland J, Mabbs R, Petty R, Fraser M, Hadley D, Patterson J, Brown SM, Rampling R. HSV1716 injection into the brain adjacent to tumour following surgical resection of high-grade glioma: safety data and long-term survival. *Gene Ther*. 2004; 11(22):1648–58. [PubMed: 15334111]
75. Johnson PA, Miyahara A, Levine F, Cahill T, Friedmann T. Cytotoxicity of a replication-defective mutant of herpes simplex virus type 1. *J Virol*. 1992; 66(5):2952–65. [PubMed: 1373198]
76. Krisky DM, Wolfe D, Goins WF, Marconi PC, Ramakrishnan R, Mata M, Rouse RJ, Fink DJ, Glorioso JC. Deletion of multiple immediate-early genes from herpes simplex virus reduces cytotoxicity and permits long-term gene expression in neurons. *Gene Ther*. 1998; 5(12):1593–603. [PubMed: 10023438]
77. Samaniego LA, Neiderhiser L, DeLuca NA. Persistence and expression of the herpes simplex virus genome in the absence of immediate-early proteins. *J Virol*. 1998; 72(4):3307–20. [PubMed: 9525658]
78. Wolfe D, Mata M, Fink DJ. A human trial of HSV-mediated gene transfer for the treatment of chronic pain. *Gene Ther*. 2009; 16(4):455–60. [PubMed: 19242524]
79. Fraefel C. Gene delivery using helper virus-free HSV-1 amplicon vectors. *Curr Protoc Neurosci*. 2007 Chapter 4, Unit 4 14.
80. Oehmig A, Fraefel C, Breakefield XO. Update on herpesvirus amplicon vectors. *Mol Ther*. 2004; 10(4):630–43. [PubMed: 15451447]
81. Hibbitt OC, Wade-Martins R. Delivery of large genomic DNA inserts >100 kb using HSV-1 amplicons. *Curr Gene Ther*. 2006; 6(3):325–36. [PubMed: 16787184]
82. Todo T. Oncolytic virus therapy using genetically engineered herpes simplex viruses. *Front Biosci*. 2008; 13:2060–4. [PubMed: 17981691]
83. Bowers WJ, Olschowka JA, Federoff HJ. Immune responses to replication-defective HSV-1 type vectors within the CNS: implications for gene therapy. *Gene Ther*. 2003; 10(11):941–5. [PubMed: 12756414]
84. Harvey BK, Chang CF, Chiang YH, Bowers WJ, Morales M, Hoffer BJ, Wang Y, Federoff HJ. HSV amplicon delivery of glial cell line-derived neurotrophic factor is neuroprotective against ischemic injury. *Exp Neurol*. 2003; 183(1):47–55. [PubMed: 12957487]

85. Klein RL, Lewis MH, Muzyczka N, Meyer EM. Prevention of 6-hydroxydopamine-induced rotational behavior by BDNF somatic gene transfer. *Brain Res.* 1999; 847(2):314–20. [PubMed: 10575102]
86. Natsume A, Mata M, Goss J, Huang S, Wolfe D, Oligino T, Glorioso J, Fink DJ. Bcl-2 and GDNF delivered by HSV-mediated gene transfer act additively to protect dopaminergic neurons from 6-OHDA-induced degeneration. *Exp Neurol.* 2001; 169(2):231–8. [PubMed: 11358438]
87. Sun M, Kong L, Wang X, Lu XG, Gao Q, Geller AI. Comparison of the capability of GDNF, BDNF, or both, to protect nigrostriatal neurons in a rat model of Parkinson's disease. *Brain Res.* 2005; 1052(2):119–29. [PubMed: 16018990]
88. Chen P, Tian J, Kovessi I, Bruder JT. Promoters influence the kinetics of transgene expression following adenovector gene delivery. *J Gene Med.* 2008; 10(2):123–31. [PubMed: 18064718]
89. Pi R, Yin W, Zheng S, Qiu P, Zhou J, Guo W, Su T, Yan G. Adenoviral mediated transfer of TIMP-3 partially prevents glutamate-induced cell death in primary cultured cortical neurons of the rat. *Brain Res Mol Brain Res.* 2004; 127(1–2):136–9. [PubMed: 15306130]
90. Boulos S, Meloni BP, Arthur PG, Bojarski C, Knuckey NW. Assessment of CMV, RSV and SYN1 promoters and the woodchuck post-transcriptional regulatory element in adenovirus vectors for transgene expression in cortical neuronal cultures. *Brain Res.* 2006; 1102(1):27–38. [PubMed: 16806110]
91. Kugler S, Lingor P, Scholl U, Zolotukhin S, Bahr M. Differential transgene expression in brain cells in vivo and in vitro from AAV-2 vectors with small transcriptional control units. *Virology.* 2003; 311(1):89–95. [PubMed: 12832206]
92. Smith RL, Traul DL, Schaack J, Clayton GH, Staley KJ, Wilcox CL. Characterization of promoter function and cell-type-specific expression from viral vectors in the nervous system. *J Virol.* 2000; 74(23):11254–61. [PubMed: 11070024]
93. Deglon N, Tseng JL, Bensadoun JC, Zurn AD, Arsenijevic Y, Pereira de Almeida L, Zufferey R, Trono D, Aebischer P. Self-inactivating lentiviral vectors with enhanced transgene expression as potential gene transfer system in Parkinson's disease. *Hum Gene Ther.* 2000; 11(1):179–90. [PubMed: 10646649]
94. Jakobsson J, Ericson C, Jansson M, Bjork E, Lundberg C. Targeted transgene expression in rat brain using lentiviral vectors. *J Neurosci Res.* 2003; 73(6):876–85. [PubMed: 12949915]
95. Kugler S, Meyn L, Holzmuller H, Gerhardt E, Isenmann S, Schulz JB, Bahr M. Neuron-specific expression of therapeutic proteins: evaluation of different cellular promoters in recombinant adenoviral vectors. *Mol Cell Neurosci.* 2001; 17(1):78–96. [PubMed: 11161471]
96. Hioki H, Kameda H, Nakamura H, Okunomiya T, Ohira K, Nakamura K, Kuroda M, Furuta T, Kaneko T. Efficient gene transduction of neurons by lentivirus with enhanced neuron-specific promoters. *Gene Ther.* 2007; 14(11):872–82. [PubMed: 17361216]
97. Peel AL, Zolotukhin S, Schrimsher GW, Muzyczka N, Reier PJ. Efficient transduction of green fluorescent protein in spinal cord neurons using adeno-associated virus vectors containing cell type-specific promoters. *Gene Ther.* 1997; 4(1):16–24. [PubMed: 9068791]
98. Oh MS, Hong SJ, Huh Y, Kim KS. Expression of transgenes in midbrain dopamine neurons using the tyrosine hydroxylase promoter. *Gene Ther.* 2009; 16(3):437–40. [PubMed: 18800154]
99. Kuroda H, Kutner RH, Bazan NG, Reiser J. A comparative analysis of constitutive and cell-specific promoters in the adult mouse hippocampus using lentivirus vector-mediated gene transfer. *J Gene Med.* 2008; 10(11):1163–75. [PubMed: 18773500]
100. Lawlor PA, Bland RJ, Mouravlev A, Young D, During MJ. Efficient gene delivery and selective transduction of glial cells in the mammalian brain by AAV serotypes isolated from nonhuman primates. *Mol Ther.* 2009; 17(10):1692–702. [PubMed: 19638961]
101. Do Thi NA, Saillour P, Ferrero L, Dedieu JF, Mallet J, Paunio T. Delivery of GDNF by an E1,E3/E4 deleted adenoviral vector and driven by a GFAP promoter prevents dopaminergic neuron degeneration in a rat model of Parkinson's disease. *Gene Ther.* 2004; 11(9):746–56. [PubMed: 14724667]
102. Liu BH, Wang X, Ma YX, Wang S. CMV enhancer/human PDGF-beta promoter for neuron-specific transgene expression. *Gene Ther.* 2004; 11(1):52–60. [PubMed: 14681697]



103. Cocho D, Belvis R, Marti-Fabregas J, Molina-Porcel L, Diaz-Manera J, Aleu A, Pagonabarraga J, Garcia-Bargo D, Mauri A, Marti-Vilalta JL. Reasons for exclusion from thrombolytic therapy following acute ischemic stroke. *Neurology*. 2005; 64(4):719–20. [PubMed: 15728300]
104. Tissue plasminogen activator for acute ischemic stroke. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group. *N Engl J Med*. 1995; 333(24):1581–7. [PubMed: 7477192]
105. Lawrence MS, Sun GH, Kunis DM, Saydam TC, Dash R, Ho DY, Sapolsky RM, Steinberg GK. Overexpression of the glucose transporter gene with a herpes simplex viral vector protects striatal neurons against stroke. *J Cereb Blood Flow Metab*. 1996; 16(2):181–5. [PubMed: 8594048]
106. Graham SH, Chen J. Programmed cell death in cerebral ischemia. *J Cereb Blood Flow Metab*. 2001; 21(2):99–109. [PubMed: 11176275]
107. Lawrence MS, Ho DY, Sun GH, Steinberg GK, Sapolsky RM. Overexpression of Bcl-2 with herpes simplex virus vectors protects CNS neurons against neurological insults in vitro and in vivo. *J Neurosci*. 1996; 16(2):486–96. [PubMed: 8551333]
108. Lawrence MS, McLaughlin JR, Sun GH, Ho DY, McIntosh L, Kunis DM, Sapolsky RM, Steinberg GK. Herpes simplex viral vectors expressing Bcl-2 are neuroprotective when delivered after a stroke. *J Cereb Blood Flow Metab*. 1997; 17(7):740–4. [PubMed: 9270490]
109. Hoehn B, Ringer TM, Xu L, Giffard RG, Sapolsky RM, Steinberg GK, Yenari MA. Overexpression of HSP72 after induction of experimental stroke protects neurons from ischemic damage. *J Cereb Blood Flow Metab*. 2001; 21(11):1303–9. [PubMed: 11702045]
110. Shimazaki K, Urabe M, Monahan J, Ozawa K, Kawai N. Adeno-associated virus vector-mediated bcl-2 gene transfer into post-ischemic gerbil brain in vivo: prospects for gene therapy of ischemia-induced neuronal death. *Gene Ther*. 2000; 7(14):1244–9. [PubMed: 10918494]
111. Sun Y, Jin K, Clark KR, Peel A, Mao XO, Chang Q, Simon RP, Greenberg DA. Adeno-associated virus-mediated delivery of BCL-w gene improves outcome after transient focal cerebral ischemia. *Gene Ther*. 2003; 10(2):115–22. [PubMed: 12571640]
112. Cao G, Xiao M, Sun F, Xiao X, Pei W, Li J, Graham SH, Simon RP, Chen J. Cloning of a novel Apaf-1-interacting protein: a potent suppressor of apoptosis and ischemic neuronal cell death. *J Neurosci*. 2004; 24(27):6189–201. [PubMed: 15240811]
113. Andsberg G, Kokaia Z, Klein RL, Muzyczka N, Lindvall O, Mandel RJ. Neuropathological and behavioral consequences of adeno-associated viral vector-mediated continuous intrastriatal neurotrophin delivery in a focal ischemia model in rats. *Neurobiol Dis*. 2002; 9(2):187–204. [PubMed: 11895371]
114. Tsai TH, Chen SL, Chiang YH, Lin SZ, Ma HI, Kuo SW, Tsao YP. Recombinant adeno-associated virus vector expressing glial cell line-derived neurotrophic factor reduces ischemia-induced damage. *Exp Neurol*. 2000; 166(2):266–75. [PubMed: 11085892]
115. Sakurai M, Abe K, Hayashi T, Setoguchi Y, Yaginuma G, Meguro T, Tabayashi K. Adenovirus-mediated glial cell line-derived neurotrophic factor gene delivery reduces motor neuron injury after transient spinal cord ischemia in rabbits. *J Thorac Cardiovasc Surg*. 2000; 120(6):1148–57. [PubMed: 11088040]
116. Yagi T, Jikihara I, Fukumura M, Watabe K, Ohashi T, Eto Y, Hara M, Maeda M. Rescue of ischemic brain injury by adenoviral gene transfer of glial cell line-derived neurotrophic factor after transient global ischemia in gerbils. *Brain Res*. 2000; 885(2):273–82. [PubMed: 11102581]
117. Arvidsson A, Kirik D, Lundberg C, Mandel RJ, Andsberg G, Kokaia Z, Lindvall O. Elevated GDNF levels following viral vector-mediated gene transfer can increase neuronal death after stroke in rats. *Neurobiol Dis*. 2003; 14(3):542–56. [PubMed: 14678770]
118. Larsson E, Mandel RJ, Klein RL, Muzyczka N, Lindvall O, Kokaia Z. Suppression of insult-induced neurogenesis in adult rat brain by brain-derived neurotrophic factor. *Exp Neurol*. 2002; 177(1):1–8. [PubMed: 12429205]
119. Gustafsson E, Andsberg G, Darsalia V, Mohapel P, Mandel RJ, Kirik D, Lindvall O, Kokaia Z. Anterograde delivery of brain-derived neurotrophic factor to striatum via nigral transduction of recombinant adeno-associated virus increases neuronal death but promotes neurogenic response following stroke. *Eur J Neurosci*. 2003; 17(12):2667–78. [PubMed: 12823474]



120. Bellomo M, Adamo EB, Deodato B, Catania MA, Mannucci C, Marini H, Marciano MC, Marini R, Sapienza S, Giacca M, Caputi AP, Squadrito F, Calapai G. Enhancement of expression of vascular endothelial growth factor after adeno-associated virus gene transfer is associated with improvement of brain ischemia injury in the gerbil. *Pharmacol Res.* 2003; 48(3):309–17. [PubMed: 12860452]
121. Zhang ZG, Zhang L, Jiang Q, Zhang R, Davies K, Powers C, Bruggen N, Chopp M. VEGF enhances angiogenesis and promotes blood-brain barrier leakage in the ischemic brain. *J Clin Invest.* 2000; 106(7):829–38. [PubMed: 11018070]
122. Shen F, Su H, Fan Y, Chen Y, Zhu Y, Liu W, Young WL, Yang GY. Adeno-associated viral-vector-mediated hypoxia-inducible vascular endothelial growth factor gene expression attenuates ischemic brain injury after focal cerebral ischemia in mice. *Stroke.* 2006; 37(10):2601–6. [PubMed: 16946160]
123. Zhao Y, Li Z, Wang R, Wei J, Li G, Zhao H. Angiopoietin 1 counteracts vascular endothelial growth factor-induced blood-brain barrier permeability and alleviates ischemic injury in the early stages of transient focal cerebral ischemia in rats. *Neurol Res.* 2010; 32(7):748–55. [PubMed: 19660197]
124. Zhu W, Fan Y, Frenzel T, Gasmi M, Bartus RT, Young WL, Yang GY, Chen Y. Insulin growth factor-I gene transfer enhances neurovascular remodeling and improves long-term stroke outcome in mice. *Stroke.* 2008; 39(4):1254–61. [PubMed: 18309153]
125. Zhu W, Fan Y, Hao Q, Shen F, Hashimoto T, Yang GY, Gasmi M, Bartus RT, Young WL, Chen Y. Postischemic IGF-1 gene transfer promotes neurovascular regeneration after experimental stroke. *J Cereb Blood Flow Metab.* 2009; 29(9):1528–37. [PubMed: 19513085]
126. Hu Q, Chen C, Khatibi NH, Li L, Yang L, Wang K, Han J, Duan W, Zhang JH, Zhou C. Lentivirus-mediated transfer of MMP-9 shRNA provides neuroprotection following focal ischemic brain injury in rats. *Brain Res.* 2010
127. Daadi MM, Li Z, Arac A, Grueter BA, Sofilos M, Malenka RC, Wu JC, Steinberg GK. Molecular and magnetic resonance imaging of human embryonic stem cell-derived neural stem cell grafts in ischemic rat brain. *Mol Ther.* 2009; 17(7):1282–91. [PubMed: 19436269]
128. Van den Haute C, Eggermont K, Nuttin B, Debyser Z, Baekelandt V. Lentiviral vector-mediated delivery of short hairpin RNA results in persistent knockdown of gene expression in mouse brain. *Hum Gene Ther.* 2003; 14(18):1799–807. [PubMed: 14670130]
129. Nielsen TT, Marion I, Hasholt L, Lundberg C. Neuron-specific RNA interference using lentiviral vectors. *J Gene Med.* 2009; 11(7):559–69. [PubMed: 19434604]
130. Michel U, Malik I, Ebert S, Bahr M, Kugler S. Long-term in vivo and in vitro AAV-2-mediated RNA interference in rat retinal ganglion cells and cultured primary neurons. *Biochem Biophys Res Commun.* 2005; 326(2):307–12. [PubMed: 15582578]
131. Ulusoy A, Sahin G, Bjorklund T, Aebischer P, Kirik D. Dose optimization for long-term rAAV-mediated RNA interference in the nigrostriatal projection neurons. *Mol Ther.* 2009; 17(9):1574–84. [PubMed: 19584816]
132. Piedrahita D, Hernandez I, Lopez-Tobon A, Fedorov D, Obara B, Manjunath BS, Boudreau RL, Davidson B, Laferla F, Gallego-Gomez JC, Kosik KS, Cardona-Gomez GP. Silencing of CDK5 reduces neurofibrillary tangles in transgenic alzheimer's mice. *J Neurosci.* 2010; 30(42):13966–76. [PubMed: 20962218]
133. Vosler PS, Sun D, Wang S, Gao Y, Kintner DB, Signore AP, Cao G, Chen J. Calcium dysregulation induces apoptosis-inducing factor release: cross-talk between PARP-1- and calpain-signaling pathways. *Exp Neurol.* 2009; 218(2):213–20. [PubMed: 19427306]
134. Cao G, Xing J, Xiao X, Liou AK, Gao Y, Yin XM, Clark RS, Graham SH, Chen J. Critical role of calpain I in mitochondrial release of apoptosis-inducing factor in ischemic neuronal injury. *J Neurosci.* 2007; 27(35):9278–93. [PubMed: 17728442]
135. Bevers MB, Ingleton LP, Che D, Cole JT, Li L, Da T, Kopil CM, Cohen AS, Neumar RW. RNAi targeting micro-calpain increases neuron survival and preserves hippocampal function after global brain ischemia. *Exp Neurol.* 2010; 224(1):170–7. [PubMed: 20298691]

136. Antonawich FJ, Federoff HJ, Davis JN. BCL-2 transduction, using a herpes simplex virus amplicon, protects hippocampal neurons from transient global ischemia. *Exp Neurol*. 1999; 156(1):130–7. [PubMed: 10192784]
137. Shirakura M, Fukumura M, Inoue M, Fujikawa S, Maeda M, Watabe K, Kyuwa S, Yoshikawa Y, Hasegawa M. Sendai virus vector-mediated gene transfer of glial cell line-derived neurotrophic factor prevents delayed neuronal death after transient global ischemia in gerbils. *Exp Anim*. 2003; 52(2):119–27. [PubMed: 12806886]
138. Shirakura M, Inoue M, Fujikawa S, Washizawa K, Komaba S, Maeda M, Watabe K, Yoshikawa Y, Hasegawa M. Postischemic administration of Sendai virus vector carrying neurotrophic factor genes prevents delayed neuronal death in gerbils. *Gene Ther*. 2004; 11(9):784–90. [PubMed: 14961067]
139. Magnoni S, Baker A, Thomson S, Jordan G, George SJ, McColl BW, McCulloch J, Horsburgh K. Neuroprotective effect of adenoviral-mediated gene transfer of TIMP-1 and -2 in ischemic brain injury. *Gene Ther*. 2007; 14(7):621–5. [PubMed: 17235293]
140. Matsuoka N, Nozaki K, Takagi Y, Nishimura M, Hayashi J, Miyatake S, Hashimoto N. Adenovirus-mediated gene transfer of fibroblast growth factor-2 increases BrdU-positive cells after forebrain ischemia in gerbils. *Stroke*. 2003; 34(6):1519–25. [PubMed: 12730553]
141. Sun HS, Jackson MF, Martin LJ, Jansen K, Teves L, Cui H, Kiyonaka S, Mori Y, Jones M, Forder JP, Golde TE, Orser BA, Macdonald JF, Tymianski M. Suppression of hippocampal TRPM7 protein prevents delayed neuronal death in brain ischemia. *Nat Neurosci*. 2009; 12(10):1300–7. [PubMed: 19734892]
142. Cannon JR, Xi G, Keep RF. Recent research on changes in genomic regulation and protein expression in intracerebral haemorrhage. *Int J Stroke*. 2007; 2(4):265–9. [PubMed: 18705926]
143. Masada T, Hua Y, Xi G, Yang GY, Hoff JT, Keep RF. Attenuation of intracerebral hemorrhage and thrombin-induced brain edema by overexpression of interleukin-1 receptor antagonist. *J Neurosurg*. 2001; 95(4):680–6. [PubMed: 11596963]
144. Zhao X, Sun G, Zhang J, Strong R, Song W, Gonzales N, Grotta JC, Aronowski J. Hematoma resolution as a target for intracerebral hemorrhage treatment: role for peroxisome proliferator-activated receptor gamma in microglia/macrophages. *Ann Neurol*. 2007; 61(4):352–62. [PubMed: 17457822]
145. Sinn DI, Chu K, Lee ST, Song EC, Jung KH, Kim EH, Park DK, Kang KM, Kim M, Roh JK. Pharmacological induction of heat shock protein exerts neuroprotective effects in experimental intracerebral hemorrhage. *Brain Res*. 2007; 1135(1):167–76. [PubMed: 17208204]
146. Lee HJ, Kim KS, Kim EJ, Choi HB, Lee KH, Park IH, Ko Y, Jeong SW, Kim SU. Brain transplantation of immortalized human neural stem cells promotes functional recovery in mouse intracerebral hemorrhage stroke model. *Stem Cells*. 2007; 25(5):1204–12. [PubMed: 17218400]
147. Lee HJ, Kim KS, Park IH, Kim SU. Human neural stem cells over-expressing VEGF provide neuroprotection, angiogenesis and functional recovery in mouse stroke model. *PLoS One*. 2007; 2(1):e156. [PubMed: 17225860]
148. Lee HJ, Park IH, Kim HJ, Kim SU. Human neural stem cells overexpressing glial cell line-derived neurotrophic factor in experimental cerebral hemorrhage. *Gene Ther*. 2009; 16(9):1066–76. [PubMed: 19554035]
149. Lee HJ, Lim IJ, Lee MC, Kim SU. Human neural stem cells genetically modified to overexpress brain-derived neurotrophic factor promote functional recovery and neuroprotection in a mouse stroke model. *J Neurosci Res*. 2010; 88(15):3282–94. [PubMed: 20818776]
150. Lee HJ, Kim MK, Kim HJ, Kim SU. Human neural stem cells genetically modified to overexpress Akt1 provide neuroprotection and functional improvement in mouse stroke model. *PLoS One*. 2009; 4(5):e5586. [PubMed: 19440551]
151. Muhonen MG, Ooboshi H, Welsh MJ, Davidson BL, Heistad DD. Gene transfer to cerebral blood vessels after subarachnoid hemorrhage. *Stroke*. 1997; 28(4):822–8. discussion 828–9. [PubMed: 9099203]
152. Yen CP, Chen SC, Lin TK, Wu SC, Chang CY, Lue SI, Jeng AY, Kassell NF, Kwan AL. CGS 26303 upregulates mRNA expression of heme oxygenase-1 in brain tissue of rats subjected to

- experimental subarachnoid hemorrhage. *J Cardiovasc Pharmacol.* 2004; 44(Suppl 1):S474–8. [PubMed: 15838352]
153. Kim DW, Im SH, Kim JY, Kim DE, Oh GT, Jeong SW. Decreased brain edema after collagenase-induced intracerebral hemorrhage in mice lacking the inducible nitric oxide synthase gene. Laboratory investigation. *J Neurosurg.* 2009; 111(5):995–1000. [PubMed: 19374494]
154. Oh S, Pluhar GE, McNeil EA, Kroeger KM, Liu C, Castro MG, Lowenstein PR, Freese A, Ohlfest JR. Efficacy of nonviral gene transfer in the canine brain. *J Neurosurg.* 2007; 107(1): 136–44. [PubMed: 17639883]
155. Kaminski JM, Huber MR, Summers JB, Ward MB. Design of a nonviral vector for site-selective, efficient integration into the human genome. *FASEB J.* 2002; 16(10):1242–7. [PubMed: 12153992]
156. Huang RQ, Qu YH, Ke WL, Zhu JH, Pei YY, Jiang C. Efficient gene delivery targeted to the brain using a transferrin-conjugated polyethyleneglycol-modified polyamidoamine dendrimer. *FASEB J.* 2007; 21(4):1117–25. [PubMed: 17218540]
157. Kim ID, Lim CM, Kim JB, Nam HY, Nam K, Kim SW, Park JS, Lee JK. Neuroprotection by biodegradable PAMAM ester (e-PAM-R)-mediated HMGB1 siRNA delivery in primary cortical cultures and in the postischemic brain. *J Control Release.* 2010; 142(3):422–30. [PubMed: 19944723]
158. Davidson BL, Breakefield XO. Viral vectors for gene delivery to the nervous system. *Nat Rev Neurosci.* 2003; 4(5):353–64. [PubMed: 12728263]
159. Nakahara Y, Gage FH, Tuszynski MH. Grafts of fibroblasts genetically modified to secrete NGF, BDNF, NT-3, or basic FGF elicit differential responses in the adult spinal cord. *Cell Transplant.* 1996; 5(2):191–204. [PubMed: 8689031]
160. Aboody KS, Brown A, Rainov NG, Bower KA, Liu S, Yang W, Small JE, Herrlinger U, Ourednik V, Black PM, Breakefield XO, Snyder EY. Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. *Proc Natl Acad Sci U S A.* 2000; 97(23):12846–51. [PubMed: 11070094]
161. Andsberg G, Kokaia Z, Bjorklund A, Lindvall O, Martinez-Serrano A. Amelioration of ischaemia-induced neuronal death in the rat striatum by NGF-secreting neural stem cells. *Eur J Neurosci.* 1998; 10(6):2026–36. [PubMed: 9753090]
162. Dayer AG, Jenny B, Sauvain MO, Potter G, Salmon P, Zraggen E, Kanemitsu M, Gascon E, Sizonenko S, Trono D, Kiss JZ. Expression of FGF-2 in neural progenitor cells enhances their potential for cellular brain repair in the rodent cortex. *Brain.* 2007; 130(Pt 11):2962–76. [PubMed: 17728358]
163. Zhu W, Mao Y, Zhao Y, Zhou LF, Wang Y, Zhu JH, Zhu Y, Yang GY. Transplantation of vascular endothelial growth factor-transfected neural stem cells into the rat brain provides neuroprotection after transient focal cerebral ischemia. *Neurosurgery.* 2005; 57(2):325–33. discussion 325–33. [PubMed: 16094163]

**Table 1**

Key features of different viral vector systems

	<b>Lentivirus</b>	<b>Adenovirus</b>	<b>AAV</b>	<b>HSV</b>
Vector genome	ssRNA	dsDNA	ssDNA	dsDNA
Insert capacity	~11kb	8~36kb	~4.7kb	40~150kb
Host chromosomal integration	Yes	No	Low frequency	No
Immunogenicity	Low	Moderate	Low	Recombinant virus: high Amplicons: low
CNS tropism	Glia and neurons	Glia > Neurons	Neurons > Glia	Neurons
Duration of expression	Months ~ years	Weeks ~ months	Months~years	Weeks~year
Clinical trial for CNS diseases	Yes	No	Yes	Yes

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