Lentiviral Vectors and Adeno-Associated Virus Vectors: Useful Tools for Gene Transfer in Pain Research

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ABSTRACT

Pain, especially chronic pain, has always been a heated point in both basic and clinical researches since it puts heavy burdens on both individuals and the whole society. A better understanding of the role of biological molecules and various ionic channels involved in pain can shed light on the mechanism under pain and advocate the development of pain management. Using viral vectors to transfer specific genes at targeted sites is a promising method for both research and clinical applications. Lentiviral vectors and adeno-associated virus (AAV) vectors which allow stable and long-term expression of transgene in non-dividing cells are widely applied in pain research. In this review, we thoroughly outline the structure, category, advantages and disadvantages and the delivery methods of lentiviral and AAV vectors. The methods through which lentiviral and AAV vectors are delivered to targeted sites are closely related with the sites, level and period of transgene expression. Focus is placed on the various delivery methods applied to deliver vectors to spinal cord and dorsal root ganglion both of which play important roles in primary nociception. Our goal is to provide insight into the features of these two viral vectors and which administration approach can be chosen for different pain researches. Anat Rec, 301:825–836, 2018. © 2017 The Authors. The Anatomical Record published by Wiley Periodicals, Inc. on behalf of American Association of Anatomists.

Key words: lentiviral vector; AAV vector; spinal cord; DRG; pain

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Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Prolonged suffering from pain affects people both physically and psychologically and is one of the main reasons for people to seek medical care and pharmacological treatment. Gene transfer to primary sensory neurons or nociceptors, which can promote the understanding of the cellular mechanisms and pathways involved in nociception, is a promising approach to study pain. Since the expression of transgene is limited at restricted sites in the nervous system, this approach is able to study specific pain-related pathways. In the early 1980s, with vectors based on the Moloney murine leukaemia virus (Mo-MLV) being developed, the concept of viral-based tools for gene delivery emerged for the first time (Mann et al., 1983). After that, viral vectors are widely used in the biological sciences, including the study of pain. At present, there are mainly five kinds of viral vectors being widely used. Among them, lentiviral (See Table 1) and adeno-associated virus (AAV) (see Table 2) vectors, which are able to transduce both dividing and non-dividing cells and to maintain long-term stable transgene expression, have gained wide interest for both fundamental and applied purpose. Here, we will summarize recent progress in the research of using lentiviral and AAV vectors for regulating gene expression in neurosciences with special emphasis on pain research.

STRUCTURE OF LENTIVIRAL AND AAV VECTORS

Lentiviral vectors are named by the fact that after the initial infection, there is a long period of time before the disease is started. Among lentiviruses, human immunodeficiency virus (HIV) 1-based vectors were the first to be developed and were most widely used in scientific research (Picanco-Castro et al., 2012). Besides, there are feline immunodeficiency virus (FIV; Da Silva et al., 2010), simian immunodeficiency virus (SIV; Liehl et al., 2007), bovine immunodeficiency virus (BIV; Berkowitz et al., 2001), equine infectious anemia virus (EIAV; Pezet et al., 2006), caprine arthritis-encephalitis virus (Mselli-Lakhal et al., 1998) and so on. Researchers' opinion on which virus is more safe and efficient is inconsistent. Generally speaking, viral vectors are combined of different plasmids, which can be classified into two parts, the packaging system required for viral particle formation and infectivity and the transfer vector consisting of cis-acting sequences sufficient to mobilize the viral genome. The transgene of interest is loaded on the latter which has been deprived of viral open reading frames (ORFs). HIV-1-based vectors are used as examples to describe the structure of lentiviral vectors. Till now, there have been three generations of lentiviral vectors, the first and the second consisting of three plasmids while the third composed of four plasmids (Picanco-Castro et al., 2012). Here we will only discuss the structure of the third generation of lentiviral vectors which are now widely used. The packaging system involves three plasmids, which provide the required trans-acting factors, namely Gag-pol, Rev, and an envelope protein, respectively. A variety of envelope proteins can be used to form pseudotypes of lentiviral vectors. Vesicular stomatitis virus protein G (VSV-G), one commonly used envelope protein, can incorporate into the viral membrane, enabling the vectors to transduce a broad range of cell types. Another plasmid is the transfer vector which contains the transgene to be delivered and contains all the cis-acting sequences required for genomic RNA production and packaging. Some researchers suggest that there is a fourth generation of lentiviral vectors, which can regulate the expression of the target gene, enormously increasing transduction efficiency and safety. The tetracycline-dependent transcriptional regulatory system is one of the best studied systems with proven efficiency both *in vitro* and *in vivo* (Sigl et al., 2014).

AAV, a member of dependovirus subfamily, is a single-stranded DNA parvovirus with a genome approximately 4.7 kb. AAV depends on other viruses such as adenovirus or herpes simplex viruses to complete its life cycle (Tseng & Agbandje-McKenna, 2014). Compared with other virus vectors, the structure of AAV vectors is relatively simple. There are three ORFs in the genome flanked by inverted terminal repeats which act as the viral origin of replication and packaging signal (Nonnenmacher & Weber, 2012). Four nonstructural proteins (Rep 78, Rep 68, Rep 52, and Rep 40) which are involved in the replication, transcription, assembly and genomic integration are encoded by the rep ORF. Three structural proteins (VP 1, VP 2, and VP 3) are encoded by the cap ORF. Besides, there is an assembly activating protein encoded by an ORF present as an alternate reading frame within the cap gene (Sonntag et al., 2010; Sonntag et al., 2011). The production of AAV vectors is through replacing rep and cap by inserting the transgene into the inverted terminal repeats with a promoter.

LENTIVIRAL VECTORS NOT DERIVED OF HIV

Pain researches utilizing lentiviral vectors not based on HIV is rare. Here, we will only discuss the application of SIV-, EIAV-, and FIV-based lentiviral vectors in pain study.

SIV-based vectors pseudotyped VSV-G, the amphotropic murine leukemia virus (MLV4070Aenv), the lymphocytic choriomeningitis virus (LCMV-GP), the Ross River virus (RRV-GP) and the rabies virus (RV-G) were used both *in vitro* and *in vivo* experiments (Liehl et al., 2007). Although each pseudotype exhibits infectivity for neuronal as well as glial cells in primary mouse brain cultures, only vectors pseudotyed with VSV-G can infect both neurons and glial cells in vivo while others preferentially transduced neuroglial cells. Besides, VSV-G pseudotypes exhibited high transduction efficiency restricted to the injected brain region while LCMV-GP and RV-G pseudotypes showed moderate efficiency and MLV4070Aenv and RRV-GP are inefficient. Moreover, deletion of the central polypurine tract sequence from the VSV-G-pseudotyped SIV transfer vector decreased the transduction efficiency *in vivo* by four-fold, and these vectors were no longer able to infect neuronal cells *in vivo*. Results above indicate that SIV vectors can be used as gene delivery vehicles for central nervous system (CNS; Liehl et al., 2007).

As to EIAV-based lentiviral vectors pseudotyped with VSV-G, they are capable of transducing both glial and neuronal cells after intraspinal administration. However, compared with HIV-based vector which transduced mainly glial cells, EIAV-based vector transfected mainly neuronal cells (Georgievska et al., 2004; Wong et al., 2004; Pezet et al., 2006).

FIV-based lentiviral vectors were used in several studies of pain. In a study delivering viral vectors to the rostral ventromedial medulla (RVM), fluorescence microscopy of brain tissue sections for green fluorescence protein (GFP) expression revealed that these vectors predominantly infected neurons located in RVM (Dull et al., 1998) The above results further prove that FIV viral vectors with VSV-G envelope transduce predominantly neurons, rather than non-neuronal cells (Alisky et al., 2000; Da Silva et al., 2010) and local neurons rather than distant neurons. Besides, there are several studies using FIV-based lentiviral vectors in the research of temporomandibular joint pain (Lai et al., 2006; Kyrkanides et al., 2007). Intraarticular injection of these vectors can infect a subset of primary sensory neurons of the ipsilateral trigeminal ganglion presumably following uptake of the lentiviral vector by peripheral nerve fibers and retrograde transportation to the nucleus. This delivery method can avoid the invasiveness and morbidity of neurosurgical procedures and address the location of pain origin instead of randomly covering an expansive area of sensory input. Transgenic animals serving as a model for long-term studies of arthritis can be created with FIV-based lentiviral vectors (Lai et al., 2006), which may potentially provide a platform for the transfer of anti-nociceptive genes for the management of temporomandibular joint pain.

DIFFERENT SEROTYPES OF AAV VECTORS AND THEIR TISSUE TROPISM

At present, more than 100 AAV genomic isolates and 13 human and non-human serotypes have been discovered (Tseng & Agbandje-McKenna, 2014). Here we will discuss six kinds of AAV vectors which have been used in the study of pain.

Compared with other serotypes, AAV serotype 2 (AAV2) vectors have been most deeply studied and most widely applied with high safety, broad host range and high transduction efficiency (Miyake et al., 2012). Besides, AAV2 vectors which can transduce neural cells efficiently and stably with minimal toxicity are excellent gene delivery tools for studies on nervous system (Tenenbaum et al., 2004). A variety of regions of the brain and spinal cord can be transduced by AAV2 vectors (Burger et al., 2005).

AAV serotype 5 (AAV5) vectors pseudotyped with viral capsids from serotype 2 have high proneness for peripheral neurons and can result in long-term and stable expression of transgene with little immunogenicity (Beutler, 2010). Besides, efficient and persistent transduction of neurons in dorsal root ganglion (DRG) can be achieved by AAV5 vectors (Mason et al., 2010).

AAV serotype 6 (AAV6) vectors can transduce sensory fiber following intravenous administration (Towne et al., 2008) and have high affinity for neurons after direct injection into the CNS (Azeredo da Silveira et al., 2009). It is typical for rAAV2/6 to result in liver transduction which reflects leakage of the vector from the injection site into the blood. However, transgene expression was not detected in liver when rAAV2/6 is administered through sciatic nerve injection (Towne et al., 2009).

AAV serotype 8 (AAV8) vectors exhibited more efficiency and wider distribution of transduced cells in the CNS than AAV2 vectors (Broekman et al., 2006). Besides, self-complementary AAV8 can be useful tools for delivering transgene into the DRG (Storek et al., 2008). In addition, the gene transfer of AAV8 to gray matter is more likely from retrograde transport through nerve terminals since it is difficult for AAV8 vector to cross blood–brain barrier (BBB) even in neonatal stage.

AAV serotype 9 (AAV9) vectors is another promising vectors for transgene delivery which can produce long-term gene expression after one-time injection (Hester et al., 2009; Lin et al., 2011). Besides, there is little immunogenicity and toxicity as to the utility of AAV9 (Foust et al., 2009). Compared with AAV8 vectors, AAV9 is more efficient in crossing the BBB and is able to delivery transgene to brain and spinal cord after intravenous vector injection (Foust et al., 2009).

However, the retrograde transport from muscle to nerve of AAV9 is much poorer than AAV8.

AAVrh20, a simian AAV serotype, is recently isolated by polymerase chain reaction-based screening of host genomes using primers specific to conserved regions of the cap gene (Asokan et al., 2012). AAVrh20 transduced the highest number of brain cells out of a panel of AAV serotypes including AAV8 when it was injected into the rat striatum (Lawlor et al., 2009). Besides, intrathecal injection of AAVrh20 resulted in a robust transduction of the primary sensory neurons in DRG, higher than that of AAV8. When injected into the rat sciatic nerve, AAVrh20 led to significantly higher transduction rates of DRG neurons compared to that of AAV1 (Pleticha et al., 2014). Therefore, AAVrh20 is likely to be a new promising AAV serotype applied in pain study.

There is differential tissue tropism among different AAV serotypes, which may be related to differential expression of cell-surface molecules required for entry of AAV vectors. As to AAV2 and AAV6, the tissue tropism is dependent on delivery methods. For sciatic nerve and intrathecal injection, greater than 90% of transduced cells are small to medium sized nociceptive neurons. Besides, nerve fibers transduced through sciatic nerve and intrathecal injection were located almost exclusively within the superficial lamina I and II of the dorsal horn (DH) whereas central terminals of DRG neurons transduced by intramuscular and subcutaneous administration were within deeper lamina (Towne et al., 2009). AAV2 intrathecally administered into spinal cord appeared to predominantly infect meningeal cells surrounding the cerebrospinal fluid space (Milligan et al., 2005). In addition, direct injection of AAV6 to DRG leads to transduction of neurons which penetrate into the spinal DH and terminate predominantly in superficial DH lamina, as well as in the dorsal columns and deeper lamina III–V (Yu et al., 2013). When AAV5 and AAV8 were intrathecally injected through lumbar puncture, GFP labeled fibers were seen predominantly in deeper lamina of DH at cervical, lumbar, and sacral level but restricted to the dorsal columns and Clarke's columns in thoracic spinal cord. Besides, AAV5 and AAV8 vectors preferentially transduced larger diameter DRG neurons in the dorsal columns, which commonly signal innocuous stimuli, but may also carry pain signals in allodynic conditions (Sun et al., 2001). In addition, the least concentration of GFP labeling was in lamina II (substantia gelatinosa) at all levels of the spinal cord, which may be due to the limited targeting of neurons smaller than 22 μm, especially IB4-binding neurons. Therefore, AAV5 and AAV8 could be used for targeting IB4-negative neuronal populations (Vulchanova et al., 2010).

DELIVERY METHODS OF LENTIVIRAL VECTORS INTO SPINAL CORD

Intrathecal Injection

Clinically, intrathecal administration of analgesics is commonly used to reduce pain at the level of the spinal cord. In pain research, intrathecal injection is also a classic method which is widely applied to deliver lentiviral vectors to the spinal cord with minor injury of the spinal parenchyma and little behavioral alterations (Luo et al., 2014; He et al., 2013; Zou et al., 2011). As early as in 1976, Yaksh and Rudy, (1976) described the implantation of intrathecal catheters for the first time. Usually, in studies using models of neuropathic pain, animal are allowed to recover for 3 days after implantation and it is necessary to monitor the general state of animals during this period (Sun et al., 2012). There are no significant changes of the general behavior of animals, such as weight, intake of food and water and reactive ability after intrathecal injection.

Intraparenchymal Injection

Intraparenchymal microinjection of lentiviral vectors which can lead to highly localized and long-term *in vivo* expression of transgene in the spinal cord is of great interest in the study of pain mechanisms at the spinal cord level (Pezet et al., 2006; Meunier et al., 2007; Meunier et al., 2008; Dominguez et al., 2010; Takasu et al., 2011). Compared with intrathecal administration, which causes diffusion of the viral suspension to the DRG, transgene expression can be anatomically restricted to the selected area of spinal cord by this method with no diffusion to contralateral spinal cord, DRG or brain stem (Meunier et al., 2007; Meunier et al., 2008). There is even no diffusion between white and grey matter. Besides, in the ipsilateral spinal cord, a large part of the lumbar enlargement (the injection site) can be infected by the vector specifically 0.5 mm along the medial/lateral axis (gray matter), 0.6–1 mm along the dorsoventral axis (DH) and 3–4 mm along the rostro-caudal axis (Meunier et al., 2008). Moreover, the method is relatively non-invasive with minor injury of the spinal parenchyma and no disturbance of the spinal cord homeostasis. Absence of significant biochemical alteration is supported by the lack of behavioral changes. In addition, several studies have found that the main target of this method is glial cell, making it an attractive tool for the study of spinal glia role in chronic pain (Meunier et al., 2007; Meunier et al., 2008; Zhang et al., 2013).

Intraneural Injection

Intraneural injection using retrograde axonal transport is another way to induce transgene expression of specific cell populations in spinal cord. According to the remote viral gene delivery model (Boulis et al., 2003), HIV-1 vecter pseudotypes bearing different envelop glycoproteins were injected into the crushed sciatic nerve of rats (Federici et al., 2009). Results showed that HIV-based lentiviral vectors pseudotyped with the Rabies PV glycoprotein might provide important vehicles for CNS targeting by peripheral injection in the treatment of pain.

Intramuscular Injection

Intramuscular injection of lentiviral vectors pseudotyped by Rabies G has demonstrated efficient transduction to spinal cord motor neurons but limited transduction to peripheral sensory neurons via retrograde transport (Mazarakis, 2001).

DELIVERY METHODS OF AAV VECTORS INTO SPINAL CORD

Intrathecal Injection

As a promising approach for studying spinal mechanism of nociception, intrathecal injection of AAV vectors could transfer genes into spinal cord with high safety and high success rate (Milligan et al., 2005; Kao et al., 2010; Vulchanova et al., 2010; Hirai et al., 2012). Besides, intrathecal injection through lumbar puncture which is applied clinically as well as for basic neuropharmacological research, offers a much less invasive route of administration for viral vector-mediated gene transfer (Storek et al., 2006; Vulchanova et al., 2010). Intravenous mannitol pretreatment can be used to facilitate the access of AAV vectors to the spinal cord (Vulchanova et al., 2010). However, the expression of transgene at spinal cord is always companied with the retrograde transport of transgene into the DRG (Kao et al., 2010).

Intraparenchymal Injection

Intraparenchymal injection into the spinal cord DH can result in significant transduction of neurons (Eaton et al., 2002; Garraway et al., 2007; Chen et al., 2010). The expression of GFP was confined to the ipsilateral DH, with minimal expression detected in the ipsilateral ventral horn or on the contralateral side. No deficits in motor function were detected in any of the injected mice (Fairbanks, 2003). Besides, cellular toxicity was not observed at both the light microscope and ultrastructural levels. Although this method is convenient for pain research, it is not ideal for clinical use.

Intramuscular Injection

In 2003, Kaspar et al., (2003) first reported retrograde transport of AAV vectors from muscle to the spinal cord.- After that, researchers have explored more serotypes of AAV vectors such as AAV1, 2, 5, 8, and 9 for retrograde transport from muscle to nerve (Federici & Boulis, 2007; Foust et al., 2008; Hollis II et al., 2008; Foust et al., 2009). After injection of AAV8 vectors to the tibialis anterior and gastrocnemius muscles of adult mice, most transduced cells were seen in white matter in the spinal cord and were found in lower part of the spinal cord. However, two months after delivery, transduced cells were detected in the upper portions such as cervical and thoracic segments besides the lower ones. Three months post-injection neurons in brain especially brain stem were also transduced (Zheng et al., 2010). Moreover, though injected into unilateral muscle, transduced cells were found in bilateral DRG, bilateral spinal cord, and brain stem.

Subarachnoid Injection

One week after subarachnoid injection, enhanced GFP (EGFP)-labeled neurons appeared in the DH and doubled by 3 weeks. Most of them were located in laminae I–III of the DH while some were located in lamina IV. Labeled cells were found in spinal cord sections up to 2 mm away from either side of the injection site; EGFP-labeled fibers could be seen up to 6 mm away from the injection site. However, none of the L4–L6 DRGs on either side of the spinal cord were labeled (Xu et al., 2003).

Intraperitoneal Injection

When AAV8 vectors were intraperitoneally injected into neonatal mice, the majority of transduced cells were located in white matter in the spinal cord. Besides, there were more transduced cells in the lower spinal cord such as lumbar segment than the higher ones such as cervical segment. In addition, DRG neurons were also infected by AAV8 vectors (Zheng et al., 2010).

Intraneural Injection and Intravenous Injection

AAV2 vectors were injected through a needle inserted into the superficial lamina of L4–5 DH along with the root entry at a depth of 220–250 μm with a micromanipulator (Cui and Bazan, 2010). Changes of behavior started only 48 hr after injection and lasted for 7 days. AAV9-mediated glial glutamate transporter-1 overexpression in spinal cord was achieved through injection via tail vein (Hester et al., 2009).

DELIVERY METHODS OF LENTIVIRAL VECTORS INTO DRG

DRG, which harbors the somata of primary sensory neurons, are thus optimally situated as one of the main subjects in pain study. The most effective method of delivering lentiviral vectors to DRG is through direct injection. Researchers have established standard techniques for delivering agents into the immediate vicinity of the DRG and injection within the DRG is well tolerated. Studies have achieved high efficiency in transgene expression highly restricted to the DRG (Takasu et al., 2011; Yu et al., 2011). A study has used a variety of modified lentivector particles with different cellular promoters, envelope glycoproteins, and viral accessory proteins to find efficient transduction of neuronal cells both *in vitro* and *in vivo* (Yu et al., 2011). Here I will briefly introduce the findings in this study. As to the promoter, the human elongation factor 1a promoter is proposed to be the most appropriate for expressing transgenes of interest in DRG neurons compared with the composite CAG promoter, the human ubiquitin C promoter and the murine phosphoglycerate kinase 1 promoter. No difference was observed in the transduction efficiency between vectors with different accessory proteins. As to envelope glycoproteins, VSV-G pseudotyped lentivector system appeared to be more efficient than rabies virus Pasteur vaccine strain, rabies virus SAD strain (RABSAD) and LCMV glycoproteins. EGFP signal was restricted to the ipsilateral DRG and mostly in neuronal cells, which indicates that the DRG microinjection method used in this report resulted in both anatomic and neuronal phenotypes selectivity but there was no difference between different subpopulations. Since this study detected the expression of EGFP in four weeks while the other in 2 weeks, time point for detection may be involved and EGFP expression following lentivector transduction in the DRG is slow in onset. Besides, lentiviral vectors were injected onto the proximal transected site of spinal nerve and transduced neurons in DRG (Ogawa et al., 2014). In conclusion, lentiviral vectors may be a valuable system for delivering target genes into DRGs to explore basic mechanisms under neuropathic pain, with the potential for future clinical use in treating chronic pain.

DELIVERY METHODS OF AAV VECTORS INTO DRG

Direct Injection

Direct injection which is well tolerated in both human and rodent subjects is a direct microinjection technique to reliably and safely deliver AAV vectors selectively into individual DRGs. This method can genetically modify segmentally restricted populations of peripheral sensory neurons, including their central processes and presynaptic terminals in the superficial lamina of the spinal cord DH (Yu et al., 2013; Fischer et al., 2014). Besides, the transfected neurons are mainly located on the ipsilateral DRG, which is superior to intrathecal delivery when restricting the spread of vector is desired (Sakai et al., 2013; Samad et al., 2013). However, some showed that the requisite surgery for viral delivery to L4 DRG is accompanied by small transient mechanical and thermal sensitization (Samad et al., 2013).

Intrathecal Injection and Intrathecal Injection through Lumbar Puncture

Intrathecal injection is a safe, easy and efficient method for delivering AAV vectors to DRG (Kao et al., 2010; Chen et al., 2014). The normal body weight, normal locomotion and somatosensory perceptions of injected animals were not disturbed. It does not damage the tissues histologically or alter the expression of unrelated endogenous mRNAs in the spinal cord and DRG (Hirai et al., 2012). Besides, this delivery method is minimally invasive, clinically applicable and is devoid of unwanted abnormalities in mechano-sensation or pain perception. Another advantage of this method is that with larger number of cells transduced, it allows better discrimination of changes in behavioral studies (Tenenbaum et al., 2004). However, it has to be noted that the needle remain at midline and be slowly inserted underneath the dura and further advanced in the subarachnoid space. Nevertheless, since AAV vectors intrathecally injected into DRG can transduce both spinal cord and DRG, the main drawback of this method is lack of specificity (Pleticha et al., 2014).

Compared with direct tissue injection methods (intraparenchymal or intraneural) or intrathecal injection through an atlanto-occipital catheter, which require requisite surgery and may cause inflammation and tissue damage, acute intrathecal delivery through lumbar puncture is minimally invasive with no requirement of surgery or catheter maintenance (Vulchanova et al.,

TABLE 1. Application of lentiviral vectors at spinal cord and DRG level in pain researches

Pain model or normal		Study sites	Delivery method	Target gene	Reference
Inflammatory pain	Induced by Freund's complete adjuvant	Spinal cord	Intrathecal injection	NF-κB/p65	Luo et al. (2014)
Neuropathic pain	Spinal nerve transection	DRG	Spinal nerve injection	Tumor necrosis factor-α	Ogawa et al. (2014)
	Chronic constriction injury	Spinal cord	Intrathecal injection	Human interleukin-10	He et al. (2013)
				NF-κBp65	Sun et al. (2012)
				Protein kinase C isoform γ	Zou et al. (2011)
			Intraparenchymal injection	Cytokine signaling 3	Dominguez et al. (2010)
				IκBα	Meunier et al. (2007)
	Spinal nerve ligation (SNL)	Spinal cord	Intraparenchymal injection	Chemokine (C-X-C motif) ligand 1	Zhang et al. (2013)
				Glial cell line-derived neurotrophic factor	Takasu et al. (2011)
				Glial cell line-derived neurotrophic factor	Pezet et al. (2006)
		DRG	Direct injection	Glial cell line-derived neurotrophic factor	Takasu et al. (2011)
Normal		Spinal cord	Intraneural injection	EGFP	Federici et al. (2009)
			Intraparenchymal injection	EGFP	Meunier et al. (2007)
		DRG	Direct injection	EGFP	Yu et al. (2013)

TABLE 2. Application of AAV vectors at spinal cord and DRG level in pain researches

Pain model or normal		Study sites	AAV serotype	Delivery method	Target gene	Reference
Inflammatory pain	Induced by formalin	DRG	AAV6 and AAV8	Intrathecal injection	Syntaxin 8	Chen et al. (2014)
Neuropathic pain	Chronic constriction injury (CCI)	Spinal cord	AAV2	Intraparenchymal injection	Brain-derived neurotrophic factor	Eaton et al. (2002)
				Intrathecal injection through lumbar puncture	Interleukin-10	Milligan et al. (2005)
		DRG	AAV6	Direct injection	MiR-7a	Sakai et al. (2013)
	Spinal nerve ligation (SNL)	DRG	AAVrh20 and AAV1	Intraneural (convection-enhanced delivery, CED)	EGFP	Pleticha et al. (2014)
			AAV8	Intrathecal injection through lumbar puncture	Prepro-β-endorphin	Storek et al. (2008)
	Spared nerve injury (SNI)	DRG	AAV2	Direct injection	Sodium channel $Na_v1.3$	Samad et al. (2013)
				Sciatic nerve injection	GTP cyclohydrolase I	Kim et al. (2009)
			AAV6	Direct injection	Ca^{2+} channel-binding domain 3 peptide	Fischer et al. (2014)
	Bennett model and Gazelius model	Spinal cord	AAV2	Intraneural injection	Agrin	Cui et al. (2010)
Normal		DRG and spinal cord	AAV2	Intrathecal injection	μ-opioid receptor	Kao et al. (2010)
			AAV5 and AAV8	Intrathecal injection through lumbar puncture	GFP	Vulchanova et al. (2010)
			AAV8	Intraperitoneal injection	GFP or LacZ	Zheng et al. (2010)
				Intramuscular injection	GFP or LacZ	Zheng et al. (2010)
			AAV9	Intrathecal injection	Superoxide dismutase-1 (SOD1)	Hirai et al. (2012)
		Spinal cord	AAV2	Intraparenchymal injection	mu-opioid receptors S196A (MOR S196A)	Chen et al. (2010)
				Subarachnoid injection	EGFP	Xu et al. (2003)
			AAV1, 2, and 5	Intrathecal injection through lumbar puncture	EGFP	Storek et al. (2006)

TABLE 2. (continued).

Pain model or normal	Study sites	AAV serotype	Delivery method	Target gene	Reference
	DRG	AAV2	Direct injection	mu opioid receptor	Xu et al. (2003)
				EGFP	Xu et al. (2003)
			Sciatic nerve injection	EGFP	Xu et al. (2003)
				mu opioid receptors	Gu et al. (2005)
			Subcutaneous injection	EGFP	Xu et al. (2003)
		AAV6	Intrathecal injection	EGFP	Towne et al. (2009)
			Sciatic nerve injection	EGFP	Towne et al. (2009)
			Subcutaneous injection	EGFP	Towne et al. (2009)
			Intramuscular injection	EGFP	Towne et al. (2009)
			Tail vein injection	EGFP	Towne et al. (2009)
		AAV6 and AAV8	Direct injection	EGFP	Yu et al. (2013)
		AAV9	Intraperitoneal injection	EGFP	Machida et al. (2013)

2010). Besides, this method can be applied in clinic as well as in basic neuropharmacological research (Storek et al., 2008). In addition, intravenous mannitol pretreatment can be used to increase the access of viral particles delivered intrathecally via lumbar puncture. Mannitol, which can control intracranial pressure and disrupt the intercellular tight junctions between endothelial cells of the CNS microvasculature, can be used intravenously to facilitate chemotherapeutic drug delivery to the CNS (Kroll & Neuwelt, 1998). The enhancement of intraparenchymal diffusion of AAV vectors have been achieved in brain, spinal, and DRG (Mastakov et al., 2002; Fu et al., 2003; Vulchanova et al., 2010).

Sciatic Nerve Injection

Although the method of direct injection is well-established, the requirement of invasive surgery of removing part of the spinal vertebra may cause unwanted nerve injury and side-effects. Besides, the efficiency of intrathecal administration is impaired by reduced effectiveness of gene transfer and poorer target specificity. Compared with these two methods, delivery through the sciatic nerve, which directly targets the axons of primary sensory or motor neurons and reaches the cell soma by means of retrograde axonal transport efficiently but less invasively, is an excellent route for gene transfer to the DRG (Gu et al., 2005; Iyer et al., 2014). Rats showed no signs of paresis and immune response after AAV vectors were injected into their sciatic nerve, suggesting that there was no permanent nerve damage associated with the procedure. Since injection of the sciatic nerve can be performed unilaterally, the non/vehicle-treated side can serve as a useful internal control for behavior or histology (Kim et al., 2009).

Convection-enhanced delivery (CED) which uses bulk flowas instead of simple diffusion to deliver macromolecules into a solid tissue has been applied to optimize vector distribution in the central and peripheral nervous system (Chen et al., 2011). Although the transduction of intraneural CED and intrathecal injection is equally high, the former can improve the anatomical selectivity of AAV vectors and the transduction was confined to the DRG of the injected peripheral nerve with no evidence of gene expression in the neighboring or contralateral DRG. However, hyperosmotic diluent did not further improve the transduction efficiency of AAV for the intraneural route (Pleticha et al., 2014).

Subcutaneous Delivery

Several studies used subcutaneous administration to deliver AAV vectors into DRG. Three weeks after subcutaneous delivery into the ventral or dorsal skin of the hind foot, there were no EGFP-positive cells in the DRGs of 3-week-old rats while EGFP-labled cells were observed in the ipsilateral DRGs of 1-week-old rats (Xu et al., 2003). Age seems to influence the efficiency of this method. Others administrating AAV6 through the middle and lateral plantar surface of the hind foot observed low efficiency of transduction in the DRGs (Towne et al., 2009). Genetical modification of AAV system can enhance the tropism of AAV to neuron cell bodies after being taken up by axons, which would further

strengthen the efficiency of subcutaneous AAV administration to DRGs.

Intramuscular Delivery

There are different results as to the efficiency of intramuscular administration of AAV vectors into the DRG. Some researchers injected AAV8 in tibialis anterior and gastrocnemius muscles in one of the hind legs of adult mice and observed long-term and efficient gene transfer in the DRG neurons more on the injected side than the contralateral side (Zheng et al., 2010). However, in another study injecting AAV6 in the triceps surae muscle of the hind limb of mice, low levels of transduction were observed in the DRG (Towne et al., 2009).

Intraperitoneal Injection

Intraperitoneal injection which provides extensive and global expression or inhibition of the target gene in all DRG neurons through a simple procedure appears to have potential for pain research. Intraperitoneal administration of AAV9 to neonatal mice lead to highly effective and specific silencing of a target gene in DRG with no major side effects and the suppression effect lasted for more than 3 months post injection (Machida et al., 2013). In another study, efficient and long-term transgene expression was observed in the DRGs of neonatal mice through intraperitoneal injection of AAV8 (Zheng et al., 2010).

Intravenous Injection

Compared with other delivery methods, administration through tail vein injection resulted in fewer eGFP-positive cells within the DRG and most of which are large non-nociceptive cells types (Towne et al., 2009).

ADVANTAGES AND DISADVANTAGES OF LENTIVIRAL

Lentiviral vectors have various advantages. Lentiviral vectors are capable of infecting a wide variety of dividing and non-dividing cells with high efficiency. Especially, lentiviral vectors have shown an enhanced proneness to transduce terminally differentiated tissues from neuronal origin (Torashima et al., 2006; Meunier & Pohl, 2009) and the normal function of infected cells is not affected both *in vitro* and *in vivo* (Torashima et al., 2006). Long term expression of the transgene can be achieved since lentiviral vectors can integrate into the host genome stably (Cockrell & Kafri, 2007). Besides, lentiviral vectors can accommodate relatively large sequences of transgenes (~12–15 kb; Kumar et al., 2001), which extends the scope of their application since most of the currently transferred genes can be cloned into lentiviral vectors. Another advantage is that lentiviral vectors have high safety with minimal immunogenicity and scarcely cause immune reaction in the host. Moreover, a wide variety of different glycoproteins can be used to form pseudotypes with lentiviral vectors, increasing its use value. In addition, lentiviral vector can be compatible with multiple transcriptional promoters, including promoters of housekeeping genes and promoters for specific cells or tissues. In addition, compared with single-stranded AAV vector, lentiviral vectors induce transgene expression more rapidly (Blesch & Tuszynski, 2007).

Despite the above advantages, lentiviral vectors also have many shortcomings. The most serious one is the uncertainty of safety. Since lentiviral vectors cannot insert transgene into specific sites, it may cause nonspecific and adverse effects. Besides, insertion mutation can happen which may lead to activation of protooncogene or inhibition of anti-oncogene, increasing the risk of cancer (Hargrove et al., 2008).

ADVANTAGES AND DISADVANTAGES OF AAV VECTORS

There are many advantages of AAV vectors. First, AAV vectors can transduce a wide range of tissue and lead to long-term and stable transgene expression in both dividing and non-dividing cell (Li et al., 2005). Second, AAV vectors are highly safe since wild-type AAV has never been shown to cause any human disease (Cao et al., 2000). Besides, AAV vectors seldom cause immune reactions and have low pathogenicity (Lentz et al., 2012). Third, because of their small size and simplicity, the production of AAV vectors is relatively easy and economical and the administration dose of AAV vectors can be higher than other virus vectors. Fourth, compared with other virus vectors, AAV vector seldom inserts into host DNA, which can reduce the possibility of insertional effects (Lentz et al., 2012).

However, there are also some disadvantages of AAV vectors. First, the packaging capacity of AAV vectors is limited to 4.7–5.0 kb in length or 4.4–4.7 kb of unique transgene sequence between the inverted terminal repeats (Grieger & Samulski, 2012), despite the different packaging limitations showed by many researchers. Second, the gene transfer efficiency of AAV vectors is impaired since the complementary DNA has to be synthesized in order to generate a dsDNA template for transcription (McCarty et al., 2001). Third, the prevalence of neutralizing antibodies in the human population increases the risks of immune reaction and decreases the efficiency of AAV vectors. Fourth, the transfection of AAV vectors to specific types of cells and tissue is low, which limits its utility.

CONCLUSION

There have been several viral systems developed as vectors for gene transfer. In this review, we have outlined characteristics of lentiviral and AAV vectors and their application in pain research. Lentiviral vectors can induce transgene expression with high efficiency and high safety. Besides, lentiviral vectors can accommodate relatively large sequences of transgenes and form pseudotypes with a wide variety of glycoproteins which enable them to be applied in a variety of areas. Nevertheless, vector integration and insertional mutagenesis have limited the use of lentiviral vectors which needs further improvement of non-integrating transduction. AAV vectors possess several promising attributes as well, including minimal toxicity, stable transduction and simplicity for scalable production. However, limited packaging capacity and relatively low transduction efficiency have been the main disadvantages of AAV vectors. Thus, although lentivital and AAV vectors are

useful tools for gene delivery in pain research, more efforts should be made in improving the efficiency and safety of these two vectors.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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