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Review

Gene therapy for the treatment of chronic peripheral nervous system pain

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ABSTRACT

Chronic pain is a major health concern affecting 80 million Americans at some time in their lives with significant associated morbidity and effects on individual quality of life. Chronic pain can result from a variety of inflammatory and nerve damaging events that include cancer, infectious diseases, autoimmune-related syndromes and surgery. Current pharmacotherapies have not provided an effective long-term solution as they are limited by drug tolerance and potential abuse. These concerns have led to the development and testing of gene therapy approaches to treat chronic pain. The potential efficacy of gene therapy for pain has been reported in numerous pre-clinical studies that demonstrate pain control at the level of the spinal cord. This promise has been recently supported by a Phase-I human trial in which a replication-defective herpes simplex virus (HSV) vector was used to deliver the human pre-proenkephalin (hPPE) gene, encoding the natural opioid peptides met- and leu-enkephalin (ENK), to cancer patients with intractable pain resulting from bone metastases (Fink et al., 2011). The study showed that the therapy was well tolerated and that patients receiving the higher doses of therapeutic vector experienced a substantial reduction in their overall pain scores for up to a month post vector injection. These exciting early clinical results await further patient testing to demonstrate treatment efficacy and will likely pave the way for other gene therapies to treat chronic pain.

Contents

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Introduction

Pain is one of the most prevalent disease complications and is now included as the fifth vital sign by most hospitals. It is estimated that 60–80 million patients within the US suffer from some form of chronic pain. As defined by the International Association for the Study of Pain, chronic pain is a severe and ever-present pain that persists for at least 3 months post initial injury or tissue damage. Chronic pain is often debilitating, leading to substantial loss of productivity and impaired quality of life. In 2005, chronic back and neck pain affected 22 million patients creating an estimated $86 billion in health care expenditures (Martin et al., 2009). Arthritis, another common cause of chronic pain, is predicted to affect 25% of the adult population by the year 2030, with 25 million experiencing activity limitations resulting from chronic pain (Hootman and Helmick, 2006). The societal burden from chronic pain patients will continue to increase as the population ages.

Nature of the chronic pain state

Chronic pain can result from inflammation and nerve damage. Nociceptive pain of an inflammatory nature is associated with a typical immune response to tissue injury or infection whereas neuropathic pain results from damage to neural structures, often in the absence of accompanying injury to non-neural tissues. Nociceptive pain is quite common and results from a variety of disease states in which short-term or long-term inflammation leads to prolonged changes in nociception. The most common incidence occurs in patients with rheumatoid or osteoarthritis, pancreatitis, or inflammatory bowel disease. Other associated conditions include interstitial cystitis (IC) or chronic pelvic pain syndrome of the bladder. Common immune mediators, such as the inflammatory cytokines interleukin-1 (IL-1), IL-6 and tumor necrosis factor-alpha (TNFα), contribute to a localized inflammatory response in the afflicted tissue or organ. They act to induce the mobilization of immune cells that amplifies their production and results in prolonged painful responses (Moalem and Tracey, 2006). The same pro-inflammatory cytokines are also secreted by glia within the spinal cord and astrocytes in response to peripheral organ and tissue inflammation, which impacts the nociceptive processes in the spinal cord (Moalem and Tracey, 2006; Sloane et al., 2009). Animal models of acute and chronic nociceptive pain have been created by injection of (i) immunogenic substances, including complete Freund's adjuvant (CFA), carrageenan and LPS, (ii) chemicals such as formalin, capsaicin, or dibutyltin dichloride, or (iii) acids like monoiodoacetate to create a model of monoarthritis or acetic acid to induce lower urinary tract pain in rats.

Neuropathic or neurogenic pain is defined as pain initiated or caused by a primary lesion or dysfunction of the nervous system. This can be the result of (i) spinal cord injury (SCI), (ii) peripheral nerve damage resulting from diabetes or other autoimmune diseases, (iii) treatment with anti-cancer drugs that affect axon integrity, or (iv) post-herpetic neuralgia (PHN). A variety of animal models mimic neuropathic pain: (i) surgical models of nerve damage, including chronic constriction injury (CCI) and spared nerve injury (SNI), (ii) streptozotocin-induced painful diabetic neuropathy and transgenic diabetic pain models, (iii) treatment with anti-cancer drugs and models of bone cancer pain established by introduction of sarcoma cells into the femur (Goss et al., 2002; Lan et al., 2010), and (iv) PHN induced by footpad injection of HSV (Kuraishi et al., 2004; Takasaki et al., 2001) or VZV (Garry et al., 2005; Hasnie et al., 2007).

The first event in pain signaling in response to inflammatory and/or mechanical damage to peripheral tissues/organs is an increase in the extracellular levels of mediators such as bradykinin, substance P (SP), ATP, hydrogen ions, histamine, prostaglandins and inflammatory cytokines such as TNFα and IL-1. It is likely that besides the peripheral signals which can induce the chronic pain response, central signals may also lead to the establishment of chronic pain, both centrally and also can manifest itself as peripheral chronic pain. The inflammatory cytokines and prostaglandins are secreted by inflammatory cells, such as resident mast cells and macrophages recruited to the initial insult *via* mast cell-released cytokines, or Schwann cells and microglia that are local to the site of nerve damage (Moalem and Tracey, 2006). Release of these molecules by the damaged tissue results in 'peripheral sensitization,' i.e. stimulation of primary afferents *via* specific receptors or ion channels sensitive to heat, mechanical impulses, protons, or cold. These stimuli activate second messenger systems, including protein kinases A and C, which results in ectopic discharge due to increased sensitivity of endogenous voltage-gated sodium and calcium channels leading to hyperalgesia, a heightened response to painful stimuli, and allodynia, pain in response to normally non-painful stimuli (Julius and Basbaum, 2001; Scholz and Woolf, 2002). In addition, the increased levels of intracellular calcium can lead to the release of neuropeptides such as SP, CGRP and neurokinin A from vesicles at the cell termini; extracellular accumulation of these factors increases their receptor occupancy in the damaged tissue, thereby amplifying the pain signal. These stimulated afferent nerve fibers carry impulses to second order neurons located within the dorsal horn of the spinal cord, the site where control and processing of the initial nociceptive signal takes place.

Pain usually occurs in two phases. The first is sharp in intensity, short in duration, very focal in nature, and is mediated by Aδ-afferents that display firing rates that correlate with the intensity of the painful stimulus. In contrast, the second phase is rather dull in intensity, displays a more prolonged duration, is not localized in nature, and is mediated by unmyelinated C-fiber afferents that display a progressive increase in their discharge rate toward second order neurons in the spinal cord (Woolf, 1996). These second-order neurons, projecting centrally to the thalamus, the dorsal reticular nucleus and periaqueductal gray, ultimately relay the signal to the cortex enabling pain perception. In addition, there are descending signaling pathways from the brain back down to the dorsal horn of the spinal cord where release of endogenous opioid peptides such as the enkephalins, ß-endorphin, dynorphins and endomorphin occurs as the body's natural pain management response (Basbaum and Fields, 1984). Chronic pain is a result of continuous or altered signaling within the activation loop. Additionally, proinflammatory agents such as prostaglandin E_2, serotonin, histamine and adenosine, and neurotrophic factors such as NGF, can induce functional changes in C-fiber afferents that can lead to hyperactivation or hyperexcitability of relatively unexcitable afferents (Gold et al., 1996). Although pain can manifest itself both centrally, such as headache, or peripherally, such as arthritis or lower back pain, this review will concentrate

only on the use of gene therapy approaches to treat peripheral forms of pain. However, many of the therapies discussed represent efforts to block pain signaling and thus are directed to treatment at the level of the spinal cord mostly *via* the expression of immune modulatory gene products that alter the host response in the case of nociceptive pain.

Current therapies for the treatment of chronic pain

Therapies for chronic pain tend to be complex as they must deal with the insult that initiates the pain response, the conditions that cause the transition from acute to chronic pain, and finally the factors that maintain the chronic state. Surgical intervention and drug therapies have been employed to treat chronic pain, but have generally met with limited success. Surgery has proven effective for some forms of chronic lower back or neck pain, but is rarely used to treat lower urinary tract pain, for example. For patients with osteoarthritis, surgical intervention may initially result in reduced pain but many of these patients eventually develop rheumatoid arthritis for which surgery is not typically prescribed. Electrical device neuromodulatory strategies that send low to high frequency modulatory impulses to the nerves involved in pain signaling using external devices, like the transcutaneous electric nerve stimulation unit, have proved effective for some patients with painful diabetic neuropathy and pain resulting from neoplasia, but have not proven successful for patients with chronic back and neck pain (Dubinsky and Miyasaki, 2010). Overall, the ability of these electrical devices to reduce chronic pain seems to be linked to the frequency employed, as lower frequency treatments display a higher failure rate than treatment at high frequency (Bennett et al., 2011). Two types of drug-mediated nerve blocks have been employed for chronic pain treatment. Trigger point injections are more local and usually involve injection of either local anesthetics or extended duration corticosteroids, while peripheral nerve block injections affect body regions. These treatment regimens have helped patients suffering from chronic pain of musculoskeletal origin, including lower back pain, whiplash, myofascial pain, and fibromyalgia. However, patients with other forms of nociceptive and neuropathic chronic pain have been refractory to these approaches.

Of all interventions for chronic pain, NSAIDs (Advil, Motrin, Aleve), other analgesics (Tylenol, Aspirin), adjuvant analgesics, and opioid analgesics are the most frequently prescribed therapies (Toblin et al., 2011). NSAIDs are the recommended first-line drugs employed in chronic pain treatment to reduce the inflammatory component *via* inhibition of cyclooxygenase, leading to a block in nociceptive signaling either at the peripheral site of injury/inflammation or within the dorsal horn of the spinal cord. The efficacy of NSAID and other non-opioid analgesic treatments is usually transient and thus these drugs are most effective against acute pain. As they are targeted at inflammatory mechanisms, they lack consistent efficacy against severe or moderate chronic pain, and additionally display unwanted side effects associated with the higher doses needed to effectively block the pain response, such as gastrointestinal and renal toxicities. However, the overall efficacy of these non-opioid analgesics can be dramatically improved by the inclusion of adjuvant analgesics, a group of drugs consisting of (i) tricyclic antidepressants such as amytriptyline, (ii) anti-epileptic drugs like carbamazepine, gabapentin and pregabalin, (iii) γ-aminobutyric acid (GABA) agonists such as baclofen, and (iv) NMDA agonists including ketamine, amantidine, dextromethorphan and memantine (Chou et al., 2009). Although adjuvant analgesics also show some signs of tolerance or risk of addiction like the opioid analgesics, they also however frequently demonstrate organ or tissue toxicities, have a narrow therapeutic window, display a ceiling effect, and generally have a sedative effect making their use in chronic pain patients with active lifestyles undesirable.

The second line of drugs includes the less potent opioid analgesics such as tramadol (Ultram), codeine, and hydrocodone (Vicodin), with or without adjuvant analgesics. They are used to treat moderate forms of chronic pain (Chou et al., 2009). The final option drugs include the potent opioids, such as morphine, methadone, levorphanol, oxycodone and fentanyl, for use against moderate to very severe chronic pain, again with or without adjuvant analgesics. The use of such prescription opioid analgesics has increased by an order of magnitude over the last 10–15 years (Ling et al., 2011), with as much as 4% of the total USA population now using opioids (Toblin et al., 2011). Hydrocodone alone was prescribed over 128 million times in 2008 (Younger et al., 2011), making it the most dispensed drug in the US ahead of lipid-regulating drugs like atorvastatin, rosuvastatin and gemfibrozil. Although the opioids display good toxicity profiles, they suffer from the complications of tolerance (i.e. dependence/addiction), abuse, and misuse/diversion. Addiction and abuse rates are low in individuals with moderate to severe chronic pain that have been prescribed opioids, but as these drugs have become more readily available, their abuse and addiction rates in the general population continue to rise (Fishbain et al., 2008).

Because opioid and non-opioid based drug therapies are efficacious in only 10–60% of patients suffering from chronic pain (Chou et al., 2009; Ling et al., 2011; Toblin et al., 2011) and risk the complications of addiction, abuse, tolerance and side effects such as nausea, constipation and toxicities associated with drug interactions, novel therapeutics for chronic pain are needed. Research into cell-based transplantation therapies was initiated in order to develop a novel non-pharmaceutical approach to treating chronic pain. The first studies employed adrenal chromaffin cells that naturally express catecholamines, met- and leu-ENK, as well as neurotrophic factors such as BDNF and NGF (Sol et al., 2005). Intrathecal (i.t.) transplantation of these cells into the subarachnoid space in formalin, SNL, CCI, cancer and arthritic pain models showed encouraging results as the grafts simply appeared to function as mini-pumps secreting their mediators into the dorsal of the spinal cord (Sol et al., 2005). However, some studies reported limited efficacy (Lindner et al., 2003). Two major concerns regarding the use of these cell grafts is that their uncontrolled growth may lead to tumor formation and that the host will mount an immune response that leads to clearance of the graft. Attempts to address the second concern with microencapsulated grafts showed that these grafts were able to both secrete catecholamines and ENK and reduce thermal hyperalgesia and mechanical allodynia levels, but their survival after 1 month was poor (Kim et al., 2009c). In addition, numerous groups have employed a variety of immortalized neuronal cells (NT2, NB69, AtT-20, RN33B, RN46A, and P19) or primary cells such as astrocytes or macrophages, transduced with either hPPE (Hino et al., 2009), POMC (Beutler et al., 1995), BDNF (Eaton et al., 1997), GAD (Eaton et al., 1999), or galanin (An et al., 2010) in cell transplant models for treating chronic pain. Again, the tumorigenic and immunogenic potential of these grafts, as well as their viability and continued release of anti-nociceptive products, represent concerns that will make the transition of these preclinical studies into human clinical trials difficult, underscoring the need for further alternatives.

Gene therapy represents a novel and targeted approach for treating chronic pain. The process employs both non-viral and viral gene transfer vectors to deliver genes encoding such candidate therapeutic products as natural opiates (ENK, POMC), effectors of neurotransmitter synthesis (GAD, Glut-1), neurotrophins or growth factors (NGF, BDNF, GDNF, VEGF, EPO, FGF2, HGF), immune modulatory factors (IL-10, IL-2, IL-4, TNFαsR, Iκβ), or anti-sense RNA to genes believed to play a role in the pain response. Many, if not all, of the cDNAs for these products are small enough in size to be readily incorporated into existing gene transfer vectors. *In vivo* gene transfer derives specificity from injection of the vectors directly into the target site or injection into peripheral tissues where natural transport mechanisms exist for bringing the vector to the target neurons or glia involved in pain modulation within the PNS. Viral vectors

for pain therapy utilize highly efficient mechanisms for transduction of neurons and glia as part of their natural biology, suggesting that sufficient numbers of cells can be transduced to achieve high levels of anti-nociceptive gene expression and attendant anti-nociceptive effects. Additionally, these gene therapeutic approaches can be combined with standard drug, physical therapy and surgical approaches to increase the overall chance of success. Since the vectors typically deliver natural gene products, the likelihood of generating tolerance or a significant immune response to the therapeutic product is minimal. However, several potential problems exist for the different non-viral and viral vector methodologies listed in Table 1 and discussed in the upcoming section, including (i) immunogenicity of the vector, (ii) vector toxicity, (iii) tumor formation as a result of viral genome integration into the host genome, (iv) cost and ease of vector production and purification, (v) overall vector safety in humans, and (vi) secondary immune responses elicited by vector re-administration.

Non-viral and viral vectors for the treatment of chronic pain

A number of methods have been tested for the delivery of non-viral plasmids to treat pain, such as injection of naked plasmid DNA, liposome- or nanoparticle-mediated delivery, and physical methods including ultrasound, electroporation, and gene gun technology. However, the most widely studied gene therapy approaches have used viral vectors for gene delivery. Viral vector gene therapy takes advantage of the natural ability of viruses to infect cells and have their genomes transported to the nucleus where their payload genes can be expressed. Viral vector systems can express transgenes for various durations, including prolonged periods of time, and some systems can express multiple genes at once or exceptionally large genes. Additionally, methods now exist to restrict or redirect the infectivity of viral vectors to specific cell types by altering viral surface proteins for exclusive recognition of target cell-specific receptors. Many of these vectors can be injected peripherally from where they will travel *via* retrograde axonal transport to the DRGs or motor neurons that innervate the site of injection. These same axonal transport mechanisms provide for the efficient delivery of viral genomes to the nucleus where transgene expression takes place. Although there are many different recombinant viral vector systems under development today for the treatment of a variety of experimental conditions, and some of these are in clinical trials, this chapter focuses on the systems that have been employed to treat chronic pain. These are based on adenovirus (AdV) [23.8% of published pain gene therapy studies according to statistics for 2010 http://www.wiley.com/legacy/wileychi/genmed/clinical/], retroviruses (RV) [20.5%], adeno-associated virus (AAV) [4.5%], herpes simplex virus (HSV) [3.3%], and lentiviruses (LV) [1.7%] (Davidson and Breakefield, 2003; Lotze and Kost, 2002); non-viral delivery systems listed include naked [17.7%] or liposome-encapsulated plasmid DNA [6.5%]. Each of these viral and non-viral vector systems has advantages and disadvantages, as summarized in Table 1. One important distinction between the different viral vectors is their ability to persist long-term and provide a sustained effect suitable for the treatment of chronic pain. Since the AdV vectors do not persist long-term, they are not ideal for the treatment of long-term chronic pain. The viral vectors that persist long-term can be further divided into two groups, those that integrate into the host genome (RV, LV, AAV) and those that persist long-term as non-integrated episomes (HSV, AAV).

Non-viral based plasmid vectors

Methods to deliver naked plasmid DNA into cells are among the simplest to achieve foreign gene expression in cells. Since they do not involve extraneous substances, the only possible chance of generating a host immune response is to the naked plasmid DNA itself *via* activation of toll-like receptors. However, the current methods for naked DNA delivery yield low transduction efficiencies, and although relatively cell-specific gene-control elements can be included, transductional specificity remains limited (Ledley, 1995). Another inherent problem with naked DNA vectors is the short duration of

Table 1
Gene delivery vectors.

Vector	Plasmid	RV	LV	AdV	AAV	HSV
1) Genome size	Varies	~10 kb	~10 kb	~40 kb	~5 kb	~150 kb
2) Payload size	Varies	++	++	+++	+	++++
(a) Size	Varies	~7 kb	~6.5 kb	~7–36 kb	~3–4.5 kb	~40 kb +
(b) Genes	Varies	1–2	1–2	1–many	1	1–many
3) Host range	Varies	Limited	Limited[a]	Broad	Broad	Broad
(a) Dividing	+	+	+	+	+	+
(b) Non-dividing	+/−	−	+	+	+	+
4) Transduction efficiency	Low	High	High	Med (10^2–10^3)	Low–med (10^3–10^5)	High (1–10)
5) Genome stability	Low	High	High	Low	High	Med–high
(a) Episomal	+	−	−	++	+	+++
(b) Integrated	+/−	++	++	−	++	−
6) Transgene expression	Med	Med	Med	High	Med	Med
(a) Short-term	+	+	+	+++	+	++
(b) Long-term	−	++	++	−	++	+/−
7) Production	Easy	Easy	Easy	Easy	Easy–hard	Hard
(a) Cell lines	−	+	+	+	+	+
(b) Kits	+	+	+	+	+	−
(c) Cost	High	Low	Low	Low	Med	Low
8) Titers (TU/mL)	[b]	10^5–10^7	10^6–10^8	10^{10}–10^{13}	10^8–10^{12}	10^9–10^{11}
9) Safety	+++	+/−	+/−	−	++/−	+
(a) Tumors	−	++	++	−	+/−	−
(b) Recomb.	−	++	++	+	+	+/−
(c) IR	++	−	−	+++++	+++	+/−
(d) Cytotoxicity	+/−	−	−	+++	−	++/−
10) Repeat Dosing	+	+/−	+/−	−	− (Eye +)[c]	++

Abbreviations: AAV, adeno-associated virus; AdV, adenovirus; HSV, herpes simplex virus; IR, immune response; kb, kilobase; LV, lentivirus; mL, milliliters; RV, retrovirus; TU, transducing units.

[a] Host range of pseudotyped LV varies with the glycoproteins employed which affects transduction efficiency.

[b] Plasmid DNA preparations in mg/mL rather than TU/mL.

[c] AAV repeat dosing has been achieved during vector delivery to the eye/retina.

transgene expression that is probably the combined result of poor transduction efficiency, low stability and persistence of the DNA, and a surprisingly strong host response to DNA. Modifications such as packaging the plasmid DNA into liposomes have shown lower immunogenicity, a higher level of transgene expression and increased (40×) transduction efficiency (Shi et al., 2003), including of DRG neurons following intrathecal injection (Wang et al., 2005), but expression has remained short term (Shi et al., 2003). Further improvements in transduction efficiencies have been seen with incorporation of the plasmid DNA into nanoparticles, but immunogenicity was variable, most likely depending on the nature of the material used to construct the nanoparticles (Belyanskaya et al., 2009). Among the physical techniques, electroporation of a recombinant plasmid encoding the natural opioid ß-endorphin resulted in expression in the rodent PNS and reduced mechanical allodynia pain measurements (Chen et al., 2008; Lin et al., 2002). Others have employed the gene gun (Chuang et al., 2003) or shockwaves (Yamashita et al., 2009) to increase the delivery of naked plasmid DNA to DRG and spinal cord neurons for treating pain. Collectively, while these physical delivery methods have shown increased *in vitro* transduction efficiencies of primary DRG neurons in culture, this enhancement has not been reproduced *in vivo* (Lin et al., 2010). A recent report (Machelska et al., 2009) employed a non-viral, non-plasmid, immunologically defined gene expression vector to treat CFA-induced chronic nociceptive pain that showed improved transduction compared with previous reports. In order to increase the specificity of non-viral gene delivery methods, NGF peptides have been used to promote binding of naked DNA complexes to TrkA-positive DRG neurons (Zeng et al., 2007) and a fragment of the tetanus toxin non-toxic subunit has been used to target the tetanus toxin receptor on DRG neurons (Oliveira et al., 2010). These modifications achieved increased transduction of DRG compared to non-neuronal cells. However, despite improvements in transduction efficiency and specificity achieved by current plasmid delivery methods, viral vectors have generally proven superior for gene delivery *in vivo*, especially to PNS neurons.

Virus-based vectors

Viral vectors provide efficient tools for gene transfer to the nervous system. Upon receptor-assisted virus entry into the cell, the viral genome is generally transported to the nucleus where it can express its resident genes, including its payload transgenes. In the case of PNS neurons, viral nucleocapsids are transported by cytoplasmic molecular motors from their point of entry at the nerve termini to the nerve cell body by retrograde axonal transport where their genomes are injected into nucleus. Viruses have evolved complex mechanisms that help them evade both innate and adaptive host cell immunity, important features to help ensure successful transduction and expression of therapeutic gene products. Many viruses are capable of persisting long-term in neurons of the PNS, either in the form of episomes (HSV, AAV) or by integration of their genome into host cell chromosomes (RV, LV, AAV) and can be provided with promoter systems capable of durable transgene expression. Although AdV vector genomes are found as non-integrated episomes, they do not persist for extended times so this class of vector is better suited to acute pain approaches yet has been used in some gene therapy approaches.

Retrovirus-based vectors

Retroviral (RV) vectors were used in the first gene transfer studies performed with cells in culture that were then transplanted back into animals in an *ex vivo* gene therapy approach. Retroviruses are enveloped viruses that contain an encapsidated dsRNA genome encoding the capsid (gag) and envelope glycoprotein (env) structural components of the virus and a reverse transcriptase (pol) (Fig. 1). Upon binding to their natural cell surface receptors, RVs enter the cell primarily by envelope fusion with the cell surface membrane although they can also enter by endocytosis. The size of RV genomes is limited by packaging contraints, allowing the incorporation of just 1–2 small transgenes (Table 1) by replacement of the structural and enzymatic genes of the virus (Fig. 1). Vectors expressing therapeutic or reporter genes can be readily generated by transfection of recombinant vector constructs into packaging cell lines that express the enzymatic and structural viral genes required for the production of new RV vector particles, but lack the RV packaging signal (Ψ). Transgenes can be expressed from the native RV promoter in the viral long terminal repeat (LTR), from other strong promoters such as the HCMV major immediate early promoter, or from cell-specific promoters.

The great majority of early gene therapy clinical trials used RV vectors based on the fact that they are easy to construct and produce with the availability of an abundance of stable packaging cell lines, display good transduction efficiencies, and yield long-term stable transgene expression as the RV genome integrates into the host DNA as part of its natural life-cycle. Although RV vectors are not immunogenic and display high therapeutic efficacy, approaches using these vectors have been hampered by two significant concerns. One is that they are unable to transduce non-dividing cells (Table 1), such as post-mitotic neurons and glia, and thus these vectors have been limited to *ex vivo* approaches with dividing cells such as Schwann cells (Girard et al., 2005). The other concern is the ability of these vectors to integrate into the DNA of the host, which can lead to disruption of normal cellular gene expression, including inactivation of tumor suppressor genes and activation of oncogenes resulting in tumorigenesis. Recently, in a clinical trial to treat a rare X-linked form of severe combined immunodeficiency, three of eleven treated patients developed T-cell leukemia due to insertions near the LMO2, BMI1, and CCND2 proto-oncogenes (Hacein-Bey-Abina et al., 2003). Further work has shown that RV vectors have a predilection to integrate at or near transcription start sites, within regions of CpG islands and DNaseI hypersensitive sites present near many proto-oncogenes (Beard et al., 2007; Derse et al., 2007), explaining the activation of the LMO2, BMI1, and CCND2 genes following infection of the large number of patient cells used in the SCID-X1 trial. Finally, the presence of endogenous RV genomes integrated at various sites within the host cell DNA allows for potential recombination between the vector genome and these endogenous RV sequences. The outcome of such recombination events, both in terms of the products they yield and the consequences for the host, has yet to be determined but are likely to be detrimental.

Lentivirus-based vectors

Lentiviral (LV) vectors, derived from human immunodeficiency virus (HIV), have received considerable interest due to their ability to infect and integrate into both dividing and non-dividing cells (Naldini et al., 1996). The structure of the LV particle is similar to that of RV (enveloped, dsRNA genome), but the virus possesses two glycoproteins responsible for its entry into cells (gp120 and gp41) and a more complex genome than standard RV (Fig. 1), encoding numerous functions in addition to the required gag, pol and env gene products. Similar to RV vectors, LV vectors are produced by transfection of a vector construct containing the therapeutic/reporter gene into packaging cell lines that provide the structural components of the virus, or by co-transfection of the vector construct with expression plasmids for gag, pol and env genes. Advantages of LV-based delivery systems (Table 1) include (i) the ease of production facilitated by commercially available kits and service companies, (ii) excellent transduction efficiencies of non-dividing cells such as PNS neurons and spinal cord glia (Finegold et al., 2001; Fleming et al., 2001; Meunier et al., 2008; Pezet et al., 2006; Wong et al., 2004), and (iii) the stability of integrated LV genomes and (iv) their extended expression pattern, which persist for many years post transduction. As

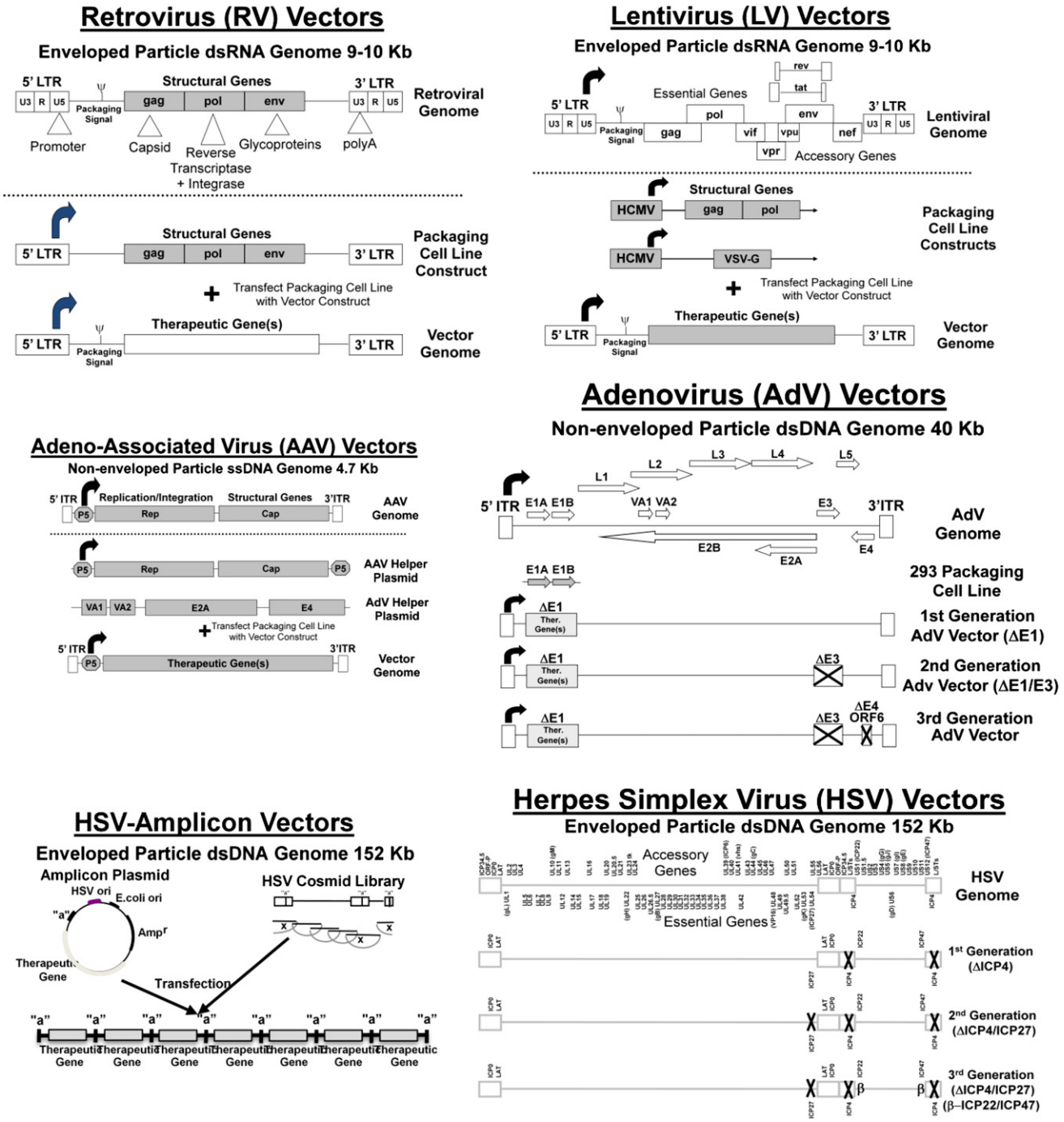

Fig. 1. Diagrams of the genomes of various viral vectors used in gene therapy approaches to treat peripheral nervous system chronic pain. The type of vector (RV, LV, AAV, AdV, HSV or HSV-amplicon) is shown along with total genome size, the positions of relevant genes, transcriptional control elements, and viral sequences involved in genome replication and packaging. Production of the replication-defective vectors generally requires special packaging or complementing cell lines to provide deleted essential genes *in trans*, as illustrated underneath each vector diagram. Abbreviations: "a", HSV packaging signal; AAV, adeno-associated virus; AdV, adenovirus; Amp^r, ampicillin resistance bacterial marker gene; ß, HSV early gene; cap, AAV capsid gene; E1–4, adenovirus early genes 1–4; *E.coli* ori, *E.coli* origin of replication; gag, group associated antigen capsid gene; HSV, herpes simplex virus; HSV-ori, HSV origin of replication; ITR, inverted terminal repeat; kb, kilobase; L1–5, adenovirus late genes 1–5; LTR, long terminal repeat; LV, lentivirus; ORF, open reading frame; Ψ, RV/LV packaging signal; pol, polymerase gene; rep, AAV replicase gene; RV, retrovirus; VA, adenovirus small viral encoded RNAs; VSV-G, vesicular stomatitis virus G envelope glycoprotein.

with standard retroviruses, LV vector integration into the host genome can lead to cell transformation, but this outcome has not been documented in human clinical trials of LV vectors to date. Unlike standard RV vectors, 50–70% of all LV integration events occur within genes rather than in upstream regulatory elements (Beard et al., 2007; Derse et al., 2007), suggesting that integration of these vectors is more likely to result in insertional inactivation of tumor suppressor genes rather than in aberrant activation of proto-oncogenes. Early LV vectors possessed a restricted host-range and suffered from low stability of the purified vector, both of which were remedied by pseudotyping of the vector with a choice of glycoproteins from other viruses (VSV, rabies, Mokola, MLV, Ebola, MuLV, measles). LV pseudotyping with rabies glycoprotein G not only increased the stability of the vector and its host-range, but also enabled retrograde

axonal transport, unlike pseudotyping with VSV-G (Fig. 1), making it possible to inject LV vectors into the periphery and achieve transduction of DRG and spinal cord neurons (Mazarakis et al., 2001; Wong et al., 2004). Since HIV, the prototypical LV, is an important human pathogen, there have been serious safety concerns regarding the use of HIV sequences in gene therapy vectors. Thus the newer LV vectors are generally designed to minimize such sequences. In addition, LV vectors have now been derived from equine infectious anemia virus, which lacks human LV sequences thereby diminishing this concern.

Adenovirus-based vectors

Adenovirus (AdV) is a non-enveloped virus possessing a dsDNA genome of approximately 40 kb in size that contains a series of early (E) genes encoding polymerase and enzymatic functions and late (L) genes for the structural capsid components (Fig. 1). AdV is a human pathogen that readily infects airway epithelial cells causing primarily a lytic infection involving lysis of infected cells, release of new virus particles, and infection of additional cells. The 1st generation, replication-deficient AdV vectors (Fig. 1) were among the first DNA viruses used in gene transfer/therapeutic approaches. These vectors were deleted for the essential E1 region of the viral genome to prevent replication, but were able to persist as episomal molecules without integration into the host genome, a crucial benefit minimizing the risk of tumorigenesis. AdV vectors display good transduction efficiencies, can mediate very high-level expression of therapeutic genes in a variety of dividing and non-dividing cell types (Table 1), including DRG neurons (Glatzel et al., 2000; Mannes et al., 1998; Watanabe et al., 2006), and can undergo retrograde transport from peripheral tissues. The 1st generation E1-deleted AdV vectors can accept moderately sized transgene inserts (~5 to 10 kb) which can be introduced with relative ease using a variety of commercially available kits and the HEK293 complementing cell line that expresses E1A and E1B, enabling the production of high-titer vector. Early generation AdV vectors continue to express several viral genes in addition to the transgene, resulting in immune recognition of infected cells and the loss of transgene expression (Varnavski et al., 2005; Yang et al., 1995). This property limits the use of these vectors in human clinical studies with the exception of cancer and vaccine trials where vector-related immunogenicity may actually be beneficial. While persistent immunogenicity of AdV vectors has hampered efforts to increase the duration of vector-mediated transgene expression by simple vector re-administration (Gonzalez et al., 2007; Yang et al., 1995), re-dosing has been possible through (i) vector PEGylation (Croyle et al., 2002), (ii) host immune suppression by pre-administration of CD40-Ig (Kuzmin et al., 2001), or (iii, #1448) vector injection into immune privileged sites such as the eye (Hamilton et al., 2006) or *in utero* (Lipshutz et al., 2000). The newest generation AdV vectors, designated "gutless" AdV (Fig. 1), have increased transgene capacity (30–36 kb) and display a reduced inflammatory response compared to previous generations (Alba et al., 2005). However, these vectors are difficult to grow and purify free of helper virus to titers suitable for certain *in vivo* studies; helper virus contamination induces similar anti-viral responses as earlier generation vectors. In general, pre-existing immunity or induction of neutralizing antibodies limits adenoviral vector readministration.

Adeno-associated virus-based vectors

Adeno-associated virus (AAV) is a relatively small, non-enveloped virus with a dsDNA genome of approximately 5-kb (Fig. 1). Since AAV is a non-pathogenic human parvovirus that is not currently associated with any human disease, it is a logical choice for development into a gene therapy vector for human trials. Similar to RV and LV vectors, replication-defective AAV vectors are produced by replacement of the early replication gene (rep) and late capsid genes (cap) with the therapeutic gene of interest (Fig. 1) and subsequent co-transfection of this construct into cells along with two plasmids, one encoding the deleted AAV gene functions (rep, cap) and another encoding the helper functions from AdV (VA1, VA2, E2A, E4) needed to propagate AAV. Several groups have employed either AdV (Chadeuf et al., 2000) or HSV as helper (Conway et al., 1997), thereby eliminating the need to co-transfect three plasmids, in an attempt to produce AAV vectors more efficiently. To the same end, others have incorporated the AAV helper gene functions into baculovirus (Urabe et al., 2002) or HSV (Clement et al., 2009). Some of the advantages (Table 1) of employing AAV include (i) the ability to infect dividing and non-dividing cells, (ii) stable genome maintenance in non-dividing cells, and (iii) very prolonged transgene expression that has proven extremely useful for a variety of pre-clinical and clinical applications involving the nervous system. One limitation of AAV is its relatively small genome size, limiting the vector payload usually to single small genes of 3–4 kb in size. This impediment has been alleviated to some degree by trans-splicing (Li et al., 2008) and the use of mini-transgene cassettes (Odom et al., 2008). Transduction of DRG neurons and spinal cord glia by AAV has been very efficient overall, regardless of the injection site or method (Fleming et al., 2001; Glatzel et al., 2000; Towne et al., 2009; Vulchanova et al., 2010). A concern with AAV vectors is the very high multiplicities required to achieve transduction that can induce DNA damage responses, vector integration and insertional mutagenesis. For example, liver transduction with high-titer AAV vectors resulted in a 33% occurrence of hepatocellular carcinoma *via* integration into chromosome 12 (Donsante et al., 2007). While it remains difficult to produce high-titer stocks consistently by co-transfection of multiple plasmids, batches of $>10^{14}$ genome copies with >90% purity are achievable (Lock et al., 2010). Like Adenovirus, AAV vectors induce neutralizing antibody responses that limit vector re-administration (Zaiss and Muruve, 2005). However, as with AdV, re-dosing has been achieved with AAV vectors by (i) reduction of the host immune response using CD40-Ig (Manning et al., 1998), or (ii) vector administration into immune-privileged sites (Li et al., 2009). In addition, serotype switching has shown promising results (Halbert et al., 2000; Riviere et al., 2006).

Herpes simplex virus-based vectors

HSV, a member of the human herpes viruses, is an enveloped virus containing a dsDNA genome of 152-kb that is composed of two segments, the unique long and unique short segment, each flanked by inverted terminal repeats (Fig. 1). The large genome encodes over 80 different gene products that are temporally expressed in three distinct waves, immediate early, early, and late. HSV genes are commonly classified as essential for virus replication in cell culture or accessory, playing a role in virus replication and pathogenesis *in vivo*. HSV has many attractive features for gene delivery to the nervous system, most importantly that it readily infects PNS neurons and can establish a latent or dormant infection in these cells as part of its natural life cycle. During latency, all of the 80+ HSV lytic genes are silent with the exception of the latency-associated transcript (LAT) locus (Stevens, 1989), which has evolved a promoter system for long-term expression during latency (Goins et al., 1994). Other advantages of HSV (Table 1) include (i) its very broad host cell range providing the opportunity to deliver genes to diverse cell populations, (ii) a very large transgene capacity capable of accommodating 30–150 kb of foreign DNA, and (iii) the persistence of the its genome as an extrachromosomal episome in non-dividing cells for the life of the host (Mellerick and Fraser, 1987). Compared to other vectors, HSV vectors are more efficient at transducing cells, especially PNS neurons, and thus fewer vector particles are required at inoculation, which minimizes toxicity and immunogenicity and decreases the likelihood of clearance of vector-transduced cells. The infectivity of HSV for neurons is many logs more efficient than that of AAV for example. One disadvantage of early generation replication-defective HSV vectors was residual vector-associated toxicity, but elimination of multiple immediate early genes from the vector genome in 3rd

Table 2
Non-opioid gene therapeutic delivery for pain treatment.

Gene	Vector	Delivery route	Model (test)	Reference
(A) Neurotrophin gene therapeutic delivery for pain treatment.				
BDNF	AAV	i.pi. spinal cord	CCI (50% change in MA, 3–4× change in TH PWL, 50% change in MH at 2–8 wpi)	Eaton et al., 2002
EPO	HSV	s.c. footpad	PDN-STZ (30% decrease in hot plate PWL)	Chattopadhyay et al., 2009
GDNF	HSV	s.c. footpad	SNL (2× change in MA at 2–6 wpi; re-dosing works; decrease in c-Fos$^+$)	Hao et al., 2003b
GDNF	LV	i.pi. spinal cord	SNL (30% change in MA and TH PWL at 3–10 dpi)	Pezet et al., 2006
HGF	HVJ-lipo	i.m. tibia cranialis	CCI (2–10× change in MA 1–6 wpi and 40% change in TH PWL at 3–42 dpi)	Tsuchihara et al., 2009
VEGF	HSV	s.c. footpad	PDN-STZ (40% change in hot plate PWL)	Chattopadhyay et al., 2005a
FGF2	AdV	i.t. spinal cord	Hemilaminectomy (20–50% change in TH PWL at 1–5 wpi)	Romero et al., 2001
NGF	AdV	i.t. spinal cord	Hemilaminectomy (20–50% change in TH PWL at 1–5 wpi)	Romero et al., 2001
(B) Neurotransmitter gene therapeutic delivery for pain treatment				
GAD65	AdV	i.n. TG	Formalin (50% change in face rubbing; bicuculline reverses)	Vit et al., 2009
GAD65	AAV	i.n. DRG	SNL (2–3× change in MA and MH at 2–10 wpi)	Lee et al., 2007
GAD65	AAV	i.n. sciatic nerve	SNK (2× change in respiration rates at 4–12 wpi)	Kim et al., 2009a
GAD67	HFV	s.c. footpad	SCI (2–3× change in MA and 40% change in PWL at 2–6 wpi; re-dosing works)	Liu et al., 2008
GAD67	HSV	s.c. footpad	PDN-STZ (3× change in TH PWL; decrease in $Na_v1.7$)	Chattopadhyay et al., 2011
GAD67	HSV	s.c. footpad	SNL (2–3× change in MA and TH PWL at 1–6 wpi; bicuculline reverses; re-dosing works)	Liu et al., 2004
GAD67	HSV	s.c. footpad	SNL (2× change in MA at 1–3 wpi)	Lee et al., 2007
GAD67	HSV	i.m. bladder	SNL (40% decrease in NVC at 3 wpi)	Miyazato et al., 2009
GAD67	HSV	i.m. bladder	SNL (80% decrease in IVP)	Miyazato et al., 2010
GLT-1	AdV	i.pi. spinal cord	SNL (3× change in MA, 30% change in PWL at 1–3 wpi)	Maeda et al., 2008
(C) Immune modulatory gene therapeutic delivery for pain treatment				
IL-10	DNA	i.t. spinal cord	Paclitaxel (3× change in MA 7–25 dpi; decreased IL-1ß, TNFα in DRG)	Ledeboer et al., 2007
IL-10	DNA	i.t. spinal cord	Acid i.m. (no effect on MA with 2 injections of plasmid DNA)	Ledeboer et al., 2006
IL-10	DNA	i.t. spinal cord	CCI (2× change in MA at 4–30 dpi needed 4 injections of plasmid DNA)	Milligan et al., 2006a
IL-10	DNA	i.t. spinal cord	CCI (10× change in MA at 3–43 dpi with 2 injections; 100 μg dose works)	Sloane et al., 2009
IL-10	DNA-lipo	i.t. spinal cord	CCI (10× change in MA at 3–43 dpi with 2 injections; 100 μg dose works)	Milligan et al., 2006b
IL-10	DNA-nano	i.t. spinal cord	CCI (10× change in MA at 1–10 wpi)	Soderquist et al., 2010
IL-10	AdV	i.t. spinal cord	CCI (10× change in MA and 2–3× change in TH PWL at 4–14 dpi; 50% decrease in IL-1ß)	Milligan et al., 2005a
IL-10	AAV	i.t. spinal cord	CCI (decrease in TH PWL) Zymosan (3–4× change in MA at 4–11 dpi)	Milligan et al., 2005b
IL-10	AAV	i.t. spinal cord	SNL (10× change in MA at 3–84 dpi)	Storek et al., 2008
IL-10	HSV	s.c. footpad	Formalin (40% decreased flinching; 2× decreased TNFα and p38 MAPK)	Zhou et al., 2008
IL-2	DNA	i.t. *vs* s.c. footpad	Carrageenan (2–6× change in TH PWL by i.t. *vs* 2–3× change in TH by footpad 1–6 dpi)	Yao et al., 2002a
IL-2	DNA-lipo	i.t. spinal cord	CCI (40% change in TH PWL at 1–7 dpi; lipo≫DNA alone; naloxone reverses)	Yao et al., 2002b
IL-2	AdV	i.t. spinal cord	CCI (10–50% change in TH PWL at 1–3 wpi)	Yao et al., 2003
IL-4	HSV	s.c. footpad	SNL (2–4× change in MA and 40% change in TH PWL 1–4 wpi; decreased c-Fos$^+$, IL-1ß, p38, PGE2)	Hao et al., 2006
Iκß	LV	i.pi. spinal cord	CCI (20% change in MA, 2× change in TH PWL at 1–3 wpi; dose-dependent decrease in IL-6, IL1ß, TNFα, iNOS)	Meunier et al., 2007
TNFαsR	HSV	s.c. footpad	SNL (3× change in MA and 20–40% change in TH PWL at 1–7 wpi; decrease in p38, IL1ß, PGE2, c-Fos$^+$; re-dosing)	Hao et al., 2007
TNFαsR	HSV	s.c. footpad	SCI (2× change in MA at 1–5 wpi)	Peng et al., 2006
TNFαsR	HSV	s.c. footpad	Morphine tolerance (20–40% change in hot plate PWL and TF at 2–7 dpi; decreased p38, IL-1ß, TNFα)	Sun et al., 2012
(D) Anti-sense gene therapeutic delivery for pain treatment				
NMDA-R1	DNA	i.t. spinal cord	Formalin (50% change in flinching; decrease c-Fos$^+$)	Lee et al., 2004
NMDA-R1	DNA-lipo	i.d. footpad	CFA (4× change in MA) Formalin (2× change in flinching)	Tan et al., 2010
NMDA-R1	AAV	i.pi. spinal cord	Formalin (2× change in MA at 3 wpi; 10% change in TH PWL; 2× decrease in flinching)	Garraway et al., 2009
NMDA-R2B	DNA-lipo	i.t. spinal cord	Formalin (2× decreased Flinching at 7–14 dpi)	Tan et al., 2005
$Ca_v1.2$	PNA	i.t. spinal cord	SNL (4 injections 40% change in MA at 2–14 dpi; 1 injection 10%)	Fossat et al., 2010
$Na_v1.7$	HSV	i.d. footpad	CFA (30% change in PWL; 50% decrease $Na_v1.7$)	Yeomans et al., 2005
$Na_v\alpha$	HSV	i.d. footpad	PDN-STZ (1.6× change in TH PWL and 5.45 change in cold acetone CA at 2 wpi; decrease in $Na_v1.7$/1.8)	Chattopadhyay et al., 2012
GABA-B1αR	HSV	i.d. footpad	Heat (30% change in TH PWL at 4 wpi)	Jones et al., 2005
μ-OR	HSV	i.d. footpad	Loperamide (10–30% change in PWL dependent on [loperamide])	Zhang et al., 2008
μ-OR	HSV	i.d. footpad	Heat + DAMGO (10–25% change in TH PWL dependent on [DAMGO])	Jones et al., 2003
3α-HSOR	DNA-lipo	i.pi. spinal cord	CCI (2× change in MA; 2–3× change in TH PWL at 8 μg dose)	Patte-Mensah et al., 2010
GCHI	AAV	sciatic nerve	SNI (2× change in MA post-SNI; 3× change in MA pre-SNI at 10–14 dpi)	Kim et al., 2009b
TLR4	DNA	i.t. spinal cord	Bone cancer (35% change in MA; 1–2× decrease APS at 3–7 dpi)	Lan et al., 2010
CGRP	HSV	i.d. footpad	Heat (2× change in TH 1–14 wpi) Capsaicin (3× change in TH PWL)	Tzabazis et al., 2007
PKCγ	LV	i.t. spinal cord	Morphine tolerance (40% change in MA; 2× change in TH PWL at 7–13 dpi; decreased IL-6, IL-1ß, TNFα)	Song et al., 2010

Table 2 (*continued*)

Gene	Vector	Delivery route	Model (test)	Reference
(E) Other gene therapeutic delivery for pain treatment				
GlyRα1	HSV	s.c. footpad	Formalin (2× change in WPS at 7 dpi 10 mM Gly, strychnine reverses)	Goss et al., 2011
		s.c. footpad	CFA (3× change in TH PWL at 1–3 dpi 100 mM Gly)	
		i.m. bladder	RTx (2× change in ICI at 1.0 mg/kg Gly)	
DN-PKCε	HSV	s.c. footpad	Capsaicin (2× change in TH PWL at 4 dpi)	Srinivasan et al., 2008

Abbreviations: AAV, adeno-associated virus; AdV, adenovirus; APS, ambulatory pain score; BDNF, brain-derived neurotrophic factor; CA, cold allodynia; Cav1.2, calcium channel, voltage-dependent, L type, alpha 1 C subunit; CGRP, calcitonin gene related peptide; CNS, central nervous system; CCI, chronic constriction injury; CFA, complete Freund's adjuvant; DAMGO, [D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin; DN, dominant-negative; dpi, days post injection; DRG, dorsal root ganglia; EPO, erythropoietin; FGF2, fibroblast growth factor-2; GABA, γ-aminobutyric acid; GDNF, glial cell-derived neurotrophic factor; GAD, glutamic acid decarboxylase; GLT, glutamate transporter; Gly, Glycine; GCHI, GTP cyclohydrolase I; HVJ, hemagglutinating virus of Japan; HGF, hepatocyte growth factor; HSV, herpes simplex virus; HSV-amp, herpes simplex amplicon; HFV, human foamy virus; HSOR, hydroxysteroid oxido-reductase; IκB, inhibitor of NF-κB-associated kinase complex; IL, interleukin; i.a., intra-articular; i.d., intradermal; i.m., intramuscular; i.pi., intraparenchymal spinal cord; i.t., intrathecal spinal cord; ICI, intercontraction interval; IL, interleukin; iNOS, inducible nitric oxide synthetase; IVP, intravesical pressure; LPS, lipopolysaccharide; LV, lentivirus; MA, mechanical allodynia; MH, mechanical hyperalgesia; Nano, nanoparticles; $Na_v1.7$, voltage-gated sodium channel; NGF, nerve growth factor; NMDA, *N*-Methyl-D-aspartate; NVC, non-voiding contractions, OR, opioid receptor; PDN, painful diabetic neuropathy; PGE, prostaglandin E; PKC, protein kinase C; PNA, peptide nucleic acid; PWL, paw withdrawal latency; RTx, resiniferatoxin; s.c., subcutaneous; SCI, spinal cord injury; SNI, spared nerve injury; SNL, spinal nerve ligation; STZ, streptozotocin; TF, tail-flick; TG, trigeminal ganglia; TH, thermal hyperalgesia; TLR, toll-like receptor; TNFαsR, tumor necrosis factor alpha soluble receptor; μg, micrograms; VEGF, vascular endothelial growth factor; wpi, weeks post injection; WPS, weighted pain score.

generation vectors (Fig. 1) significantly reduced cytotoxicity (Krisky et al., 1998). These highly defective mutant vectors readily establish persistence in sensory neurons and in other cell types, thus providing ideal backbones for the expression of therapeutic genes. HSV possesses a natural promoter system that is uniquely active during latency when all the other viral promoters are repressed (Goins et al., 1994). This latency-active promoter system has been used to achieve long-term expression of transgenes in sensory neurons of the PNS and CNS (Chattopadhyay et al., 2005b; Goins et al., 1999; Palmer et al., 2000; Perez et al., 2004; Puskovic et al., 2004), key targets for chronic pain gene therapies. Moreover, the LAP2 component of the latency-active promoter system can be used in combination with other promoters, including strong or cell-specific promoters, to achieve high-level, long-term transgene expression. Another advantage of HSV vectors is that they can be injected directly into a specific dermatome of tissue where rapid uptake is achieved and infection occurs by retrograde axonal transport in sensory nerves that innervate the site of injection. For example, inoculation of the skin of the footpad with HSV vectors expressing pre-proenkephalin (ENK) where local expression of ENK opiate peptides inhibits nociceptive or neuropathic pain signaling in various animal models of acute and chronic pain (see Table 2).

As an alternative to using genomic full-length replication defective HSV vectors, several laboratories have employed what have been termed HSV-amplicon vectors. These vectors (Fig. 1) are simple to generate as they are based on cloning of the desired therapeutic gene of interest into an amplicon plasmid that contains an HSV origin of replication (Ori_S) and the HSV cleavage and packaging ("a") sequence needed for incorporation of the amplicon DNA into newly synthesized virus particles (Epstein, 2009). The generation of new HSV particles is achieved by co-transfection of cells with the amplicon plasmid and either a series of overlapping HSV cosmids or an HSV genome on a bacterial artificial chromosome (HSV-BAC), both deleted for the HSV "a" sequences to prevent their incorporation into newly synthesized virus particles. This procedure yields concatermerized amplicon DNA packaged into particles with HSV structural proteins and surface glycoproteins expressed from the cosmid or BAC helper sequences. Because of the small size of amplicon plasmids and the overwhelming size of the HSV genome, amplicon vectors can accommodate large amounts of foreign sequences. However, since their production relies on co-transfection, it has been challenging to produce large-scale, high-titer batches of stable, pure amplicon vectors; typical titers are in the order of 10^6–10^8 infectious particles/mL compared to 10^{10}–10^{12} PFU/mL for replication defective HSV vectors. Other concerns are significant residual toxicity and the host immune response to amplicon vectors which many groups have tried to remedy (Ryan and Federoff, 2009). Nevertheless, HSV-amplicon vectors have been effectively applied in the treatment of animal models of chronic pain (Zou et al., 2011).

Gene therapy approaches for the treatment of chronic pain

Neurotrophic/growth factor gene therapy

Growth factor delivery comprised most of the original gene therapeutic studies for pain and were based on many of the initial cell therapy approaches in which transplanted cells expressing neurotrophins such as BDNF, NGF or GDNF, either naturally or induced, were employed to reverse pain signaling following intrathecal or intraparenchymal transplant into the spinal cord of rodents. Both non-viral (HVJ-liposomes) and viral (AAV, LV and HSV) vectors have been employed to transfer different neurotrophins or growth factors [e.g. vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), or erythropoietin (EPO)], to DRG primary neuron nociceptors or cells of the spinal cord. Direct injection of AAV-BDNF (Eaton et al., 2002) or HVJ-liposomes-HGF (Tsuchihara et al., 2009) into the parenchyma of the spinal cord improved both mechanical allodynia and thermal hyperalgesia in the CCI neuropathic pain model (see Table 2A), and similar results were observed following HSV- or LV-mediated delivery of GDNF in the SNL neuropathic pain model (Hao et al., 2003b; Pezet et al., 2006) or on administration of HSV vectors expressing EPO (Chattopadhyay et al., 2009), or VEGF (Chattopadhyay et al., 2005a) in painful diabetic neuropathy models. Additionally, AdV vector-mediated expression of FGF2 and NGF reduced thermal hyperalgesia in animals experiencing hemilaminectomy, whereas vector expressing the L1 adhesion molecule failed to alter nociceptive behavior (Romero et al., 2001). Although the exact mechanism(s) accounting for these changes to neuropathic nociception still remain(s) to be resolved, the initial role of these neurotrophins or growth factors in increasing neuronal survival can not alone account for the changes observed in the different animal models of neuropathic pain. Some studies have suggested that increased expression of NGF, BDNF and GDNF causes changes in CGRP and SP levels (Wang et al., 2003) and in the levels of ATF3 observed in animal models of neuropathic pain (Pezet et al., 2006). Moreover, increased expression of growth factors may result in lower levels of cytokine synthesis and release in neuropathic and nociceptive pain models (Jia et al., 2009), all consistent with roles for these factors besides their growth promoting activities.

Opioid peptide gene therapy

Initial approaches for the delivery of genes encoding natural opioid peptides, such as the human pre-proenkephalin (hPPE) gene yielding both met- and leu-ENK, or the pro-opiomelanocortin (POMC) gene

Table 3
Opioid gene therapeutic delivery for pain treatment.

Gene	Vector	Delivery route	Model (test results)	Reference
Enkephalin	DNA-GG	i.m. bladder	Capsaicin (16–43% change in ICI at 4–7 dpi)	Chuang et al., 2005
Enkephalin	HSV	i.d. footpad	Capsaicin (2× change in PWL)	Wilson et al., 1999
Enkephalin	HSV	s.c. footpad	Formalin (50%, 25, 10% decrease in WPS at 7, 14, and 28 dpi; re-dosing gives 60% reduction; naloxone reverses)	Goss et al., 2001
Enkephalin	HSV	i.d. footpad	CFA-arthritis (2× change in MA, 3× increased mobility)	Braz et al., 2001
Enkephalin	HSV	i.m. bladder	Capsaicin (12% decrease in ICI; naloxone reverses)	Yoshimura et al., 2001
Enkephalin	HSV	s.c. footpad	Bone cancer (90%/60% decrease in APS at 1/3 wpi)	Goss et al., 2002
Enkephalin	HSV	s.c. footpad	SNL (2× change in MA 2–6 wpi; naloxone reverses; 10× decrease in morphine ED_{50}; re-dosing works)	Hao et al., 2003a
Enkephalin	HSV	i.d. footpad	PTx (2× change in PWL at 1–6 wpi)	Yeomans et al., 2004
Enkephalin	HSV	i.d. vibrissal pad	CCI (15× change in MA at 1–6 wpi)	Meunier et al., 2005
Enkephalin	HSV	i.d. footpad	Capsaicin (3× change in PWL 2–20 wpi; naloxone reverses)	Yeomans et al., 2006
Enkephalin	HSV	Pancreas	Pancreatitis-DBTC (2× decrease in Rearing; decrease in spinal cord c-Fos^+)	Lu et al., 2007
Enkephalin	HSV	i.a. knee joint	CFA-arthritis (10× change in MA, 20% change in TH PWL; decrease in spinal cord c-Fos^+)	Lu et al., 2008
Enkephalin	HSV	i.a. knee joint	CFA-arthritis (20% change in TH PWL)	Pinto et al., 2008
Enkephalin	HSV	Pancreas	Pancreatitis-DBTC (30% change in TH PWL at 3–9 wpi)	Yang et al., 2008
Enkephalin	HSV	s.c. footpad	SNL-morphine (2–3× change in MA; decreased jumping)	Hao et al., 2009a
Enkephalin	HSV	i.m. bladder	Capsaicin/RTx (20% change in ICI, 20% decrease in licking, 60% decrease in freezing)	Yokoyama et al., 2009
Enkephalin	HSV	i.t. spinal cord	CCI (30% change in MA and PWL at 1–5 wpi)	Zou et al., 2011
ß-Endorphin	DNA	i.t. spinal cord	Formalin (2× decrease in flinching, TF, PWL)	Lee et al., 2003
ß-Endorphin	DNA-MIDGE	s.c. footpad	CFA (2× change in MA)	Machelska et al., 2009
ß-Endorphin	DNA-EP	i.t. spinal cord	CCI (2–3× change in MA and in TH PWL 7–14 dpi)	Lin et al., 2002
ß-Endorphin	DNA-EP	i.t. spinal cord	CCI (2× change in MA and TH PWL at 2–10 dpi)	Wu et al., 2004
ß-Endorphin	DNA-EP	i.t. spinal cord	CCI (2× change in MA and TH PWL at 7–14 dpi)	Chen et al., 2008
ß-Endorphin	DNA-SW	i.m. gluteus maximus	CFA (2× change in MA, 20–30% change in TH PWL)	Yamashita et al., 2009
ß-Endorphin	DNA-GG	i.m. bladder	Acetic Acid (2× change in ICI)	Chuang et al., 2003
ß-Endorphin	DNA-GG	s.c. footpad	Formalin (40% decrease in flinching, 10% change in TH PWL)	Lu et al., 2002
ß-Endorphin	AdV	i.t. + i.pi. spinal cord	Carrageenan (2× change in PWL)	Finegold, et al., 1999
ß-Endorphin	AAV	i.t. spinal cord	SNL (3× change in MA at 1–3 wpi; naloxone reverses)	Storek et al., 2008
Endomorphin	HSV	s.c. footpad	SNL (2–3× change in MA and TH PWL at 1–5 wpi)	Wolfe et al., 2007
Endomorphin	HSV	s.c. footpad	CFA + formalin (4–5× change in MA, 20–30% change in PWL, 2× decrease in TF at 1–3 wpi)	Hao et al., 2009b
μ-OR	AAV	sciatic nerve	CFA + morphine tolerance (30% change in PWL)	Gu et al., 2005
μ-OR	AAV	i.pi. spinal cord	Morphine tolerance (TF, PWL; 50% change in morphine ED_{50})	Chen et al., 2007
μ-OR	AAV	i.pi. spinal cord	Morphine tolerance (TF; decrease in morphine ED_{50})	Kao et al., 2010
μ-OR	HSV	i.d. footpad	Loperamide (20% change in TH PWL)	Zhang et al., 2008

Abbreviations: AAV, adeno-associated virus; AdV, adenovirus; APS, ambulatory pain score; CNS, central nervous system; CCI, chronic constriction injury; CFA, complete Freund's adjuvant; DBTC, dibutyltin dichloride; dpi, days post infection; ED_{50}, 50% effective dose; EP, electroporation; GG, gene gun; HSV, herpes simplex virus; ICI, intercontraction interval; i.a., intra-articular; i.d., intra-dermal; i.m., intra-muscular; i.pi., intra-parenchyma spinal cord; i.t., intrathecal spinal cord; MA, mechanical allodynia; MIDGE, non-viral/non-plasmid minimalistic immunologically defined gene expression; OR, opioid receptor; PTx, pertussis toxin; PWL, paw withdrawal latency; RTx, resiniferatoxin; SW, shockwave; SNL, spinal nerve ligation; s.c., sub-cutaneous; TF, tail-flick; TH, thermal hyperalgesia; wpi, weeks post infection; WPS, weighted pain score.

responsible for the production of adrenocorticotropic hormone, melanocyte-stimulating hormone, and ß-endorphin, were also based on transplantation studies with cells that either naturally express these products (Kim et al., 2009c; Sol et al., 2005) or had been modified to express hPPE (Hino et al., 2009) or POMC (Beutler et al., 1995). Pohl and coworkers first showed that a *tk*-defective HSV recombinant injected subcutaneously in the paw transduced DRG neurons for enkephalin expression (Antunes Bras et al., 1998), and the Glorioso and Wilson laboratories (Table 3) first used hPPE-expressing HSV vectors in formalin- and capsaicin-induced nociceptive pain models (Wilson et al., 1999). These initial studies were followed by additional work with HSV vectors in similar models (Goss et al., 2001; Yeomans et al., 2006; Yokoyama et al., 2009; Yoshimura et al., 2001). Furthermore, replication-defective genomic HSV vectors expressing ENK (Table 3) have been tested in animal models of arthritis (Braz et al., 2001; Lu et al., 2008; Pinto et al., 2008), pancreatitis (Lu et al., 2007; Yang et al., 2008), and pertussis toxin-induced models of nociceptive pain (Yeomans et al., 2004), as well as in the SNL (Hao et al., 2003a, 2009a), CCI (Zou et al., 2011) and bone cancer (Goss et al., 2002) models of neuropathic pain.

Endorphin (Table 3) has been primarily delivered by plasmid DNA vectors to treat models of arthritic pain (Machelska et al., 2009; Yamashita et al., 2009), formalin- or acetic acid-induced nociceptive pain (Chuang et al., 2003; Lee et al., 2003; Lu et al., 2002), and the CCI model of neuropathic pain (Chen et al., 2008; Lin et al., 2002; Wu et al., 2004). In addition, viral vectors such as AAV and AdV (Table 3) have been used to express endorphins for the treatment of SNL neuropathic (Beutler et al., 2005) and carrageenan-induced nociceptive (Finegold et al., 1999) pain. Although an actual endomorphin gene has not been identified, HSV vectors expressing endomorphin peptides instead of met- and leu-ENK from an engineered hPPE construct (Wolfe et al., 2007) proved efficacious (Table 3) in the treatment of CFA-induced arthritis (Hao et al., 2009b) and the SNL model of neuropathic pain (Wolfe et al., 2007). The final gene therapy applications using opioid therapy (Table 3) have followed a slightly different approach involving expression of the mu opioid receptor, using AAV (Chen et al., 2007; Gu et al., 2005; Kao et al., 2010) or HSV (Zhang et al., 2008) vectors where the pain response in different models could be altered by adjusting the morphine regimen showing that ectopic production of soluble mediators (opioid peptides or neurotrophins) is not essential to elicit a suppressive host response to pain signals.

Neurotransmitter-based gene therapy

Inhibitory amino acid neurotransmitters such as GABA and glycine play a crucial role in modulating synaptic circuits in the CNS and are

major inhibitors of neuropathic pain signaling in the dorsal horn of the spinal cord, as suggested by the fact that GABA agonists such as baclofen are effective in blocking neuropathic pain in some patients. The enzyme glutamic acid decarboxylase (GAD), present as two different molecular weight forms, GAD67 and GAD65, converts the neurotransmitter glutamate into GABA and thereby represents a potential pain modulatory effector for use in gene therapy applications. Delivery of the more membrane-associated GAD isoform (GAD65) using AAV (Kim et al., 2009a; Lee et al., 2007) or AdV (Vit et al., 2009) resulted in reduced pain in both a neuropathic SNL and a formalin-induced pain model (Table 2B). The more cytosolic GAD67 isoform, delivered by a human foamy virus vector (Liu et al., 2008) or an HSV vector (Chattopadhyay et al., 2011; Lee et al., 2007; Liu et al., 2004; Miyazato et al., 2009, 2010), was effective in altering nociception in various SCI models (Table 2B), whether injected into the footpad or the bladder. Like studies (Goss et al., 2001) where the opioids were expressed (Table 3), some of the GAD67 expression therapies could be re-administered to achieve even greater nociceptive effects than observed following the first injections (Liu et al., 2004, 2008). Finally, it was also possible to use bicuculline to reverse the pain response achieved with GAD65/67 gene therapy (Liu et al., 2004; Vit et al., 2009) similar to that seen using naloxone in the opioid gene therapy studies (Goss et al., 2001; Hao et al., 2003a; Yeomans et al., 2006; Yoshimura et al., 2001).

Glutamate is an excitatory amino acid neurotransmitter whose production is stimulated following inflammation or peripheral nerve injury. It is released by the DRG nerve termini in the dorsal horn of the spinal cord and affects signaling *via* second order neurons projecting to the brain. Removal of glutamate from the synaptic cleft within the dorsal horn of the spinal cord can play a major role in modulating the pain response by keeping its concentration within a range that prevents over-excitability, a process that may result in the transition from acute to chronic pain. The glutamate transporter Glt-1 is down regulated in SNL and CCI models of neuropathic pain. When expressed in spinal cord astrocytes, it helps maintain the balance of extracellular glutamate and thus represents a promising candidate for pain gene therapy. Indeed, delivery of Glt-1 using AdV vectors (Table 2B) helped mitigate pain in both the SNL model of neuropathic pain and the carrageenan model of nociceptive pain (Maeda et al., 2008), providing further evidence that proper maintenance of excitatory and inhibitory amino acid neurotransmitters is crucial to blocking chronic pain.

Immuno-modulatory molecule gene therapy

The role of the immune system in causing and exacerbating pain is a crucial component in the establishment and maintenance of the chronic pain state. It not only naturally alters nociceptive pain states, but it also plays a role in neuropathic pain. Numerous modulators of inflammation have been employed to alter pain, including inhibitory cytokines such as IL-2, IL-4, and IL-10, and modulators of inflammatory cytokines such as TNFα soluble receptor (TNFαsR), IL-6Ra and IκB, affecting the activity of TNFα, IL-6 and NFκB, respectively. The majority of this type of gene therapy application (Table 2C) has focused on the delivery of the anti-inflammatory cytokine IL-10 using plasmid DNA in CCI (Milligan et al., 2006a, 2006b; Sloane et al., 2009; Soderquist et al., 2010) and paclitaxel-induced (Ledeboer et al., 2007) models of neuropathic pain as well as acid-induced nociceptive pain (Ledeboer et al., 2006). In addition, IL-10 delivery has been performed with viral vectors such as AAV (Milligan et al., 2005b; Storek et al., 2008) and AdV (Milligan et al., 2005a) in CCI and SNL neuropathic pain models and with HSV in formalin-induced pain (Zhou et al., 2008). Both plasmid and viral vector delivery methods have achieved reduced pain levels (Table 2C). IL-2 expressed from non-viral plasmid DNA vectors (Yao et al., 2002a, 2002b) or AdV (Yao et al., 2003) has also been efficacious in CCI and carrageenan models of neuropathic and nociceptive pain (Table 2C), and HSV vector-based expression of IL-4 (Table 2C) was shown to reduce pain in an SNL model of neuropathic pain following direct sub-cutaneous injection into the footpad (Hao et al., 2006).

TNFαsR expressed from HSV vectors (Table 2C) alleviated chronic neuropathic pain in SCI and SNL models following footpad injection (Hao et al., 2007; Peng et al., 2006; Sun et al., 2012). Many genes that modulate inflammation or alter the pain response are up-regulated by the transcription factor NFκB, making this protein a potential target for gene therapy approaches using molecules such as IκB, a natural inhibitor of NFκB. LV vector-mediated expression of IκB has been shown to have a dramatic effect on mechanical allodynia in both the CCI neuropathic and LPS nociceptive pain models (Table 2) when used to transduce astrocytes *via* intraparenchymal injection of the spinal cord (Meunier et al., 2007). Because all of the immune modulators mentioned here had an effect on neuropathic as well as nociceptive pain, it is evident that inflammation plays a role in the induction or maintenance of chronic neuropathic pain. Many of the studies expressing the various immune modulatory gene products (Hao et al., 2006, 2007; Ledeboer et al., 2007; Meunier et al., 2007; Milligan et al., 2005a; Sun et al., 2012; Zhou et al., 2008) demonstrated that expression of the therapeutic molecule not only led to a change in nociceptive behavior, but it also caused a reduction in many of the inflammatory mediators such as TNFα, IL-6, IL-1ß, p38-MAPK, iNOS that may account for the alleviated pain responses observed. Like prior studies using other gene therapeutics for treating chronic pain, the HSV vector expressing the TNFα soluble receptor was able to re-establish a block in the pain response observed in SNL rats, and this response could be reversed by naloxone treatment.

Anti-sense-based gene therapy

Vectors expressing anti-sense versions of genes involved in the induction or maintenance of pain were first tested by the Wilson and Yeomans labs. While activation of NMDA-R by glutamate leads to pain induction, both non-viral plasmid DNA vectors (Lee et al., 2004; Tan et al., 2005, 2010) and AAV (Garraway et al., 2009) expressing anti-sense NMDA-R in nociceptive pain models led to a reduction in flinching and mechanical allodynia (Table 2D). Similarly, HSV anti-sense vectors to the GABA-R (Jones et al., 2005) or mu opioid receptor (Jones et al., 2003; Zhang et al., 2008) altered nociceptive pain paw withdrawal latency (Table 2D). Additionally, HSV or protein-nucleic acid vectors expressing sodium or calcium channel anti-sense constructs were efficacious in a CFA nociceptive pain model (Yeomans et al., 2005) or a SNL neuropathic pain model (Fossat et al., 2010). A very recent study using HSV vectors expressing a $Na_V\alpha$ anti-sense construct led to reduced expression of both $Na_V1.7$ and $Na_V1.8$ that resulted in changes in both thermal hyperalgesia and cold allodynia in streptozotocin-treated rats with painful diabetic neuropathy (Chattopadhyay et al., 2012). HSV vectors expressing anti-sense to CGRP (Tzabazis et al., 2007) reduced thermal hyperalgesia in a heat-induced pain model and paw withdrawal latency in a capsaicin-induced model of nociceptive pain (Table 2D). Injection of plasmid DNA vectors expressing anti-sense to TLR4 (Lan et al., 2010) or alpha-hydroxysteroid oxido-reductase (Patte-Mensah et al., 2010) resulted in reduced mechanical allodynia and ambulatory pain scores in behavioral studies of neuropathic pain following direct injection into the spinal cord (Table 2D). Anti-sense to GTP cyclohydrolase I delivered by AAV in a SNI model of neuropathic pain led to decreased mechanical allodynia after sciatic nerve injection (Kim et al., 2009b). Finally, LV mediated PKCγ anti-sense expression (Song et al., 2010) altered morphine-induced mechanical allodynia and paw withdrawal latency (Table 2D) following intrathecal injection into the sub-arachnoid space. Considering that the anti-sense approaches have employed vectors expressing complete or partial anti-sense constructs rather than regions that have previously

been determined to reduce expression of the target gene, the overall results have been quite remarkable.

TRPV1 modulator gene therapy

The vanilloid receptor TRPV1 is a pro-nociceptive cationic channel activated by the binding of protons, capsaicin, endovanilloids and noxious heat, indicating an important role in pain signaling. Several nociceptive pain mediators can up-regulate expression of protein kinase C-epsilon (PKCε), which phosphorylates TRPV1 in response to binding of capsaicin or other TRPV1 agonists. HSV vectors expressing a dominant-negative form of PKCε (Table 2E) increased the paw withdrawal latency following footpad injection of vector in a capsaicin-induced model of nociceptive pain (Srinivasan et al., 2008).

Recent work by the Glorioso lab has developed a two-vector HSV co-infection system to select for novel TRPV1-inhibitory genes (Srinivasan et al., 2007). In the presence of capsaicin, replication of a TRPV1-expressing HSV vector was blocked as a result of cell death due to calcium overload. However, replication could be rescued by expression of a dominant-negative form of TRPV1 from a second HSV vector. This observation holds promise for the use of HSV-based cDNA libraries (Wolfe et al., 2010) to select for genes that negatively regulate TRPV1 or other ion channels involved in pain signaling.

Clinical gene therapy trials for pain

The overall goal of all the previously described animal model pain studies was to identify a vector and therapeutic gene combination that lead to a consistent reduction in pain behavior in order to take that application to the clinic. However, going from small scale animal studies to even Phase-I clinical trials requires the consideration of critical issues such as (i) vector production/purification scale-up, (ii) delivery/administration route, (iii) vector formulation, (iv) toxicity of the vector and/or therapeutic transgene, (v) recombination of the vector with endogenous viral sequences present within the host target cells, (vi) reactivation of resident wild-type virus within the host tissue, (vii) the generation of a innate or adaptive host response to the vector and/or therapeutic transgene, as well as (viii) overall safety of the therapeutic regimen.

NP2 was developed as a 3rd generation replication-defective HSV vector for expression of ENK from the hPPE gene. Based on considerable pre-clinical data for this and earlier-generation HSV-ENK vectors in a variety of pain models (Braz et al., 2001; Goss et al., 2001, 2002; Hao et al., 2003a, 2009a; Wilson et al., 1999; Yeomans et al., 2004, 2006), NP2 was approved for a dose-escalation Phase-I human clinical trial in patients suffering from moderate to severe intractable pain due to primary or metastatic cancer (Fink et al., 2011). The vector was injected ten times (100 μL/injection) directly into the duratome innervating the pain-afflicted region at the site of the tumor. Study exclusion criteria included patients experiencing HSV-related disease, those who were undergoing chemo- or radiation-therapy or had surgery within the last 6 months, and patients with immunodeficiencies or who were seropositive for HIV, hepatitis C and B viruses. Four patients received the lowest dose (10^7 plaque forming units, PFU), while three each received the middle (10^8 PFU) or highest (10^9 PFU) dose. All were monitored for severe adverse events (SAEs), other abnormal functions, and their NRS pain scores were monitored for 4 weeks post-injection (wpi).

The first key observation from this Phase-I study was that none of the ten patients experienced a treatment-related SAE (Fink et al., 2011), attesting to the safety of this application using replication-defective HSV to deliver ENK as safety is the primary endpoint readout of a Phase-I trial. However, efficacy of the therapeutic approach was also seen in this Phase-I trial with a limited patient population. Patients receiving the lowest dose (10^7 PFU) showed a reduction in their NRS pain scores from ~8 down to 6 over the 4-week period. Moreover, those injected with the middle dose (10^8 PFU) had their scores drop from 9 to 1 during the first two weeks, followed by an increase to level 4 by 4 weeks. Finally, the highest-dose patients (10^9 PFU) displayed the greatest efficacy, showing NRS values of 8 reduced to 1 at 2 weeks, with subsequent increases to a maximum score of 2 by the end of the study (Fink et al., 2011). The pattern of alteration in the patient NRS pain scores closely resembled that the kinetics observed in pre-clinical studies where shut-off of the HCMV promoter driving expression of the hPPE gene resulted in transient ENK expression waning between 2 and 4 weeks (Goss et al., 2001, 2002). In summary, no SAEs were reported using the NP2 vector system and even in this limited patient population size this trial showed encouraging efficacy results for chronic pain sufferers. This in turn has led to a Phase-II trial to assess the maximum tolerated dose in a placebo-controlled dose-escalation study that will also examine re-administration of the vector in some patients. Positive efficacy data results in this Phase-II study would enable the assessment of the NP2 vector in an even greater patient population to more effectively assess efficacy. Additionally, a Phase I–II trial for patients with painful diabetic neuropathy will soon be initiated by Dr. David Fink and Diamyd Inc. using another replication-defective HSV vector expressing GAD67, based on the pre-clinical studies employing HSV-GAD67 vectors (Chattopadhyay et al., 2011; Lee et al., 2007; Liu et al., 2004; Miyazato et al., 2009, 2010). Finally, Dr. Linda Watkins and colleagues in conjunction with Xalud Therapeutics Inc. are preparing to initiate a Phase-I pain trial using the XT-101 vector system expressing the IL-10 therapeutic gene.

Summary and future directions

The exciting results that have been observed using both non-viral plasmid and various viral vectors expressing a variety of gene products (neurotrophins, opioids, neurotransmitters, immune modulators, and anti-sense to numerous products) in both nociceptive and neuropathic pain animal models have led to a Phase-I human trial using a replication-defective HSV vector expressing ENK. Although this first gene therapy trial for chronic pain has been a success, there are ways in which gene transfer and expression can be improved. For example, direct vector injection into animals generally results in transduction of target and non-target cells, dependent on the vector used (Table 1) and route of administration. Current studies therefore include efforts to restrict the expression of anti-nociceptive gene products to the proper target cells using both transductional and transcriptional targeting.

Another area for improvement is the duration and regulation of expression of the therapeutic product. In HSV vectors, combination of the latency promoter LAP2 that provides for long-term expression (Goins et al., 1994) with other promoters that are normally shut-off between 14 and 28 days (Chattopadhyay et al., 2005b; Goins et al., 1999; Palmer et al., 2000; Perez et al., 2004; Puskovic et al., 2004), has resulted in high-level, long-term transgene expression, but this has yet to be tested in animal models of pain and the system is uncontrolled in the fact that once the promoter becomes active, it can not be shut down. While LAP2 in combination with cell-specific promoter elements may be responsive to the normal regulatory mechanisms of the host cell, the therapist again has no control over the levels or duration of transgene expression following vector delivery. Thus there is a need for novel approaches where it is possible to activate the therapy only in the presence of when the patient is treated with a safe, non-toxic drug that only affects the target therapy. We have recently engineered a 3rd generation replication-defective HSV vector that expresses the ligand-regulated glycine receptor (GlyR), a chloride ion channel that responds to the inhibitory amino acid neurotransmitter glycine (Goss et al., 2011). Since GlyR is not normally present on DRG neurons, transduction of DRG neurons with the HSV-GlyR vector should enable activation of the channel only in

the presence of the exogenously administered ligand glycine. The HSV-GlyR vector was tested in the formalin-induced and CFA-induced nociceptive pain models following sub-cutaneous footpad injection and in a resiniferatoxin (RTx)-induced nociceptive pain model after vector injection into the bladder (Table 2E). In all models, the injection of vector alone or glycine alone had no effect on normal nociception. However, administration of glycine at various times post vector injection reduced paw withdrawal latency in the formalin and CFA models and reduced bladder hyperactivity in RTx-treated animals. Moreover, the level of anti-nociception achieved correlated with the doses of both HSV-GlyR vector and drug, demonstrating a method for regulating the pain response by drug addition. One complication in advancing this therapeutically is the presence of GlyR on central projection neurons restricting the use of a pill or systemic glycine injection to regulate the nociceptive response. However, a mutant form of GlyR has been described that is no longer responsive to glycine administration but responds to a related ligand, ivermectin (Lynagh and Lynch, 2010), an FDA-approved drug in widespread use since the late 1980s to eradicate a devastating parasitic infection in tropical countries (Omura, 2008). Ivermectin can be systemically administered since it has no effect on endogenous GlyR located centrally and would only act on vector-expressed mutant GlyR. Additionally, in combination with vector re-targeting, this system where the addition of a drug results in activation of the pain therapy can potentially be used to identify different PNS neuronal subtypes that contribute to either nociceptive or neuropathic pain, or to acute *versus* chronic pain.

Abbreviations

"a"	HSV packaging signal;
AAV	adeno-associated virus
AdV	adenovirus
APS	ambulatory pain score
Amp^r	ampicillin resistance bacterial marker gene
ß	HSV early gene
BDNF	brain-derived neurotrophic factor
CA	cold allodynia
cap	AAV capsid gene
Cav1.2	calcium channel, voltage-dependent, L type, alpha 1C subunit
CCI	chronic constriction injury
CFA	complete Freund's adjuvant
CGRP	calcitonin gene-related peptide
CNS	central nervous system
DAMGO	[D-Ala^2, N-$MePhe^4$, Gly-ol]-enkephalin
DBTC	dibutyltin dichloride
dpi	days post infection
DN	dominant-negative
dpi	days post injection
DRG	dorsal root ganglia
ED_{50}	50% effective dose
E1–4	adenovirus early genes 1–4
E.coli ori	*E.coli* origin of replication
ENK	enkephalin
env	envelope gene(s)
EP	electroporation
EPO	erythropoietin
FGF2	fibroblast growth factor-2
GABA	γ-aminobutyric acid
GDNF	glial cell-derived neurotrophic factor
GAD	glutamic acid decarboxylase
gag	group associated antigen or capsid gene(s)
GCHI	GTP cyclohydrolase I
GG	gene gun
GLT	glutamate transporter
Gly	Glycine
gp	glycoprotein
HCMV	human cytomegalovirus
HFV	human foamy virus
HGF	hepatocyte growth factor
HIV	human immunodeficiency virus
hPPE	human pre-proenkephalin
HSOR	hydroxysteroid oxido-reductase
HSV	herpes simplex virus
HSV-ori	HSV origin of replication
HVJ	hemagglutinating virus of Japan
IC	interstitial cystitis
ICI	intercontraction interval
IκB	inhibitor of NF-κB-associated kinase complex
IL	interleukin
i.a.	intra-articular
i.d.	intra-dermal
i.m.	intra-muscular
i.pi.	intra-parenchyma spinal cord
i.t.	intrathecal spinal cord
iNOS	inducible nitric oxide synthetase
IR	immune response
ITR	inverted terminal repeat
IVP	intravesical pressure
kb	kilobase
L1–5	adenovirus late genes 1–5
LAT	HSV latency-associated transcript
LPS	lipopolysaccharide
LTR	long terminal repeat
LV	lentivirus
MA	mechanical allodynia
MIDGE	non-viral/non-plasmid minimalistic immunologically defined gene expression
MH	mechanical hyperalgesia
mL	milliliters
MLV	murine leukemia virus
MuLV	Moloney murine leukemia virus
Nano	nanoparticles
$Na_v1.7$	voltage-gated sodium channel
NGF	nerve growth factor
NMDA	*N*-Methyl-D-aspartate
NVC	non-voiding contractions, OR, opioid receptor
ORF	open reading frame
Ψ	RV/LV packaging signal
PDN	painful diabetic neuropathy
PFU	plaque forming unit
PGE	prostaglandin E
PKC	protein kinase C
PHN	post-herpetic neuralgia
PNA	peptide nucleic acid
pol	polymerase gene
POMC	pro-opiomelanocortin
PTx	pertussis toxin
PWL	paw withdrawal latency
rep	AAV replicase gene
RTx	resiniferatoxin
RV	retrovirus
SAE	severe adverse event
SCI	spinal cord injury
SNI	spared nerve injury
SW	shockwave
SNL	spinal nerve ligation
s.c.	sub-cutaneous
SP	substance P
STZ	streptozotocin
TF	tail-flick
TG	trigeminal ganglia

TH	thermal hyperalgesia
TLR	toll-like receptor
TNFαsR	tumor necrosis factor alpha soluble receptor
Trk	tyrosine receptor kinase
TRPV1	transient receptor potential vanilloid-1
TU	transducing units
μg	micrograms
VA	adenovirus small viral encoded RNAs
VEGF	vascular endothelial growth factor
VSV-G	vesicular stomatitis virus G envelope glycoprotein
VZV	Varicella zoster virus
wpi	weeks post infection
WPS	weighted pain score.

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