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Gene Therapy for the Treatment of Diabetic Neuropathy

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Abstract

Neuropathy is a common, untreatable complication of both type 1 and type 2 diabetes. In animal models peptide neurotrophic factors can be used to protect against the development of neuropathy, but the combination of short half-life and off-target effects of these potent pleiotropic peptides has limited translation to human therapy. Gene transfer is a promising strategy that might circumvent these limitations. In this essay we review the basic methods of gene transfer and the preclinical data in rodent models that support the utility of this approach in the treatment of diabetic neuropathy. The path to a clinical applications and potential pitfalls in developing gene therapy for the treatment of diabetic neuropathy are considered.

Introduction

Diabetic polyneuropathy is a major complication of both insulin dependent and non-insulin dependent diabetes mellitus. Tight control of glycemia [1] is the only treatment that has been demonstrated to improve the signs and symptoms of neuropathy, but the improvement achieved by even years of tight control is incomplete. Many pathogenic mechanisms that might be responsible for the damage which develops in diabetic nerve have been identified. These include, but are not limited to, increased polyol pathway activity, nonenzymatic glycation of nerve proteins, endoneurial hypoxia, oxidative stress and mitochondrial damage. In peripheral nerve, conjugated dienes are increased and hydroperoxides reduced in the nerve of STZ diabetic rats, glutathione levels and superoxide dismutase activity reduced. An argument has been made that nuclear factor κ B (NF- κ B) is of central importance in the pathogenesis of neuropathy. But despite decades of work, it would be fair to conclude that no approaches directed at these potential primary mechanisms has produced a therapy to prevent the progression of diabetic neuropathy, much less to reverse established disease. For example, an extensive literature documents the effects of aldose reductase inhibitors on the progression of neuropathy in animal models, but the results of human trials have failed to support the utility of this therapy in treatment of the human disease.

Neurotrophic factors in diabetic neuropathy

There is limited evidence to suggest a role for loss of neurotrophic support in the pathogenesis of diabetic neuropathy. For example, primary sensory neurons express insulin receptors and are responsive to insulin treatment *in vitro* and *in vivo*. C-peptide levels are reduced in type 1 diabetes and a C-peptide receptor has been identified in peripheral nerve. Similarly, the level of insulin like growth factor (IGF-1) is reduced in rats with both type 1 and type 2 diabetes and serum IGF-1 levels are reduced in adult patients with insulin-dependent or non-insulin dependent diabetes. But it is difficult to separate the direct effects

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of insulin deficiency in type 1 diabetes from the effects of other metabolic consequences of insulin deficiency, and it is not at all clear that any of these deficiencies are of primary importance in the development of neuropathy.

The role of target-derived factors in promoting the survival of specific cell populations during development dates back to observations by Shorey that removal of skeletal muscle targets results in a reduction in the number of brachial motor neurons in the adult and by Hamburger and Levi-Montalcini that nerve growth factor (NGF) is important for the survival of sensory and sympathetic neurons. Most traditional neurotrophic factors are expressed to high levels transiently during development, preventing programmed cell death during critical periods of development, but there is substantial evidence to suggest that in adult animals neurotrophic factors can be used to protect neurons from a variety of toxic insults, and speed recovery from injury. The mechanism of actions of these trophic factors, their distribution *in vivo*, and the regional and cellular distribution of the cognate receptors is beyond the scope of this brief summary but the response of mature neurons to these factors provides a potential therapeutic intervention to ameliorate the effects of cell injury or neurodegeneration.

NGF is probably the most extensively studied trophic factor in diabetic neuropathy. NGF levels are reduced in diabetic nerve [2,3], and though NGF receptor expression is normal in STZ-diabetic nerve there is a marked decrease in receptor saturation and concomitant reduced retrograde axonal transport of NGF. Systemic administration of NGF to STZ diabetic rats ameliorates tail-flick measures of pain threshold [4], reduces the fall in substance P and calcitonin gene related peptide (CGRP) levels in the ganglion [4], and increases CGRP mRNA in the DRG [5]. Extensive preclinical testing in rodents demonstrated that NGF administered systemically is effective in preventing the progression of diabetic neuropathy. Other trophic factors that have been shown to have a protective effect in diabetic neuropathy models in rodents include: brain derived neurotrophic factor (BDNF) [6]; neurotrophin-3 [7,8]; ciliary neurotrophic factor [9,10]; interleukin 6 [11]; glial cell derived neurotrophic factor [12,13]; sonic hedgehog [14]; IGF [15] C peptide [16]; fibroblast growth factor [17]; and erythropoietin [18]. A detailed review was published recently [19].

Results suggesting a protective effect of NGF in patients with diabetic neuropathy were obtained in a phase 2 human trial [20], but in the following phase 3 prospective randomized control trial of efficacy NGF was found to be ineffective in preventing the progression of neuropathy [21]. A number of potential pitfalls can be identified that may have contributed to the failure of NGF treatment in this trial. The most obvious is the dose-related effect. In animal studies of various neuropathies NGF was administered in doses ranging 3–5 mg/kg [4,22], but because of dose-related side effects that occurred with doses > 1 µg/kg in the Phase I trial, rhNGF was given to patients at 0.1 µg/kg twice-weekly [21]. The potential therapeutic benefit is further compromised very short half-life of the peptide *in vivo*, which is < 10 min in rodents [23].

Gene transfer

Administration of other trophic factors to treat patients with neuropathy is likely to be beset by the same problems that have impeded the translation of NGF treatment from animal models to patients. The combination of off-target effects of the potent pleiotropic peptides limits the maximum dose that can be administered; in combination with the short half-life this makes it very difficult to achieve therapeutic efficacy by systemic administration. As a result, interest has grown in the potential to use gene transfer methods to achieve continuous therapeutic peptide expression. Two different gene therapy strategies have been used. In

one, injection of a gene transfer vector into non-neural tissues such as muscle is used to effect the continuous systemic delivery of the trophic factor. In the second, viral vectors are used to transduce primary sensory neurons in the dorsal root ganglion to effect the local release of the trophic factor in the nervous system.

There are two different classes of gene transfer vectors. Non-viral vectors are created by complexing cationic lipids with plasmid DNA. This approach is technically simpler than viral vectors and even though gene delivery is relatively inefficient some success has been reported. The most effective gene delivery vehicles are created by modification of viruses, organisms that have evolved elaborate strategies for the transfer their genetic material into host cells. Adenovirus, adenoassociated virus-based (AAV) and herpes simplex virus-based vectors (HSV) have shown promise in rodent models of neuropathy and will be described in some detail.

Recombinant adenovirus vectors are typically engineered to lack the tumor-causing gene E1a and propagated in complementing cell lines constructed to express E1a. The deletion of E1a prevents the virus from replicating in most cell types, although low level expression of other viral genes can be detected. "Gutless" adenoviral vectors deleted for all viral functions can be propagated by co-transfection with wild-type adenovirus, with the defective vector separated from the helper virus after propagation. Adenoviral vectors produces very high levels of transgene expression, which is a positive, but expression tends to be transient and the virus is highly immunogenic. In human applications adenoviral vectors have been used largely as a platform for vaccine delivery, or for treatment of cancer where the immune response may be used to advantage.

AAV was initially discovered as a contaminant of preparations of adenovirus. Natural AAV infection is not known to cause disease and AAV-based vectors are widely used in phase 1 and phase 2 human gene transfer trials. AAV is a small (22 nm) single-stranded DNA virus that can accommodate up to 5 kb of foreign genetic sequences. AAV vectors have conventionally been propagated using a triple transfection method in which one plasmid containing packaging signals and transgene, a second plasmid expressing the AAV *replicase* and *capsid* functions, and a third coding for the required adenovirus helper functions are co-transfected. More recently, methods involving bacterial artificial chromosomes have been developed to increase the efficiency of AAV vector production. In vitro, the wild type AAV genome integrates into a specific site on chromosome 19; in vivo, integration is relatively inefficient and requires concatemerization of the vector genome so that it may take several weeks to achieve maximum transgene expression following vector injection.

HSV is particularly well-suited for delivery of genes to the dorsal root ganglion (DRG) [24,25]. The wild-type virus has a natural tropism for peripheral sensory neurons of the DRG where it is capable of establishing life-long persistence in a latent state in which viral genomes persist as intranuclear episomal elements for the lifespan of the host. The lifelong persistence of latent genomes in trigeminal ganglion without the development of sensory loss or histological damage to the ganglion attests to the effectiveness of these natural latency mechanisms. Recombinant vectors that are entirely replication-defective retain the ability to establish persistent quiescent genomes in neurons, but are unable to replicate (or reactivate) in the nervous system [24].

The HSV particle consists of a nucleocapsid surrounded by an envelope containing glycoproteins essential for virus attachment and penetration into cells. Between the capsid and the envelope is a protein matrix (tegument) containing a number of structural proteins, including VP16, that act in concert with cellular transcription factors to activate HSV immediate-early (IE) gene promoters, and the UL41 (virion host shutoff [vhs]) gene product,

which shuts off host protein synthesis. The HSV genome contains 152 kb of linear, double-stranded DNA encoding > 80 gene products [26] and consisting of two segments a unique long (UL) and unique short (US) segment each of which is flanked by inverted repeats containing important IE and latency genes. The viral genes are almost entirely found as contiguous transcribable units, making their genetic manipulation relatively straightforward. In wild-type infection, the virus is transmitted by direct contact, replicating initially in epithelial cells of skin or mucous membranes. Second-generation virions are taken up by sensory nerve terminals and carried by retrograde axonal transport to the neuronal perikaryon in DRG, where viral DNA is injected through a modified capsid penton into the nucleus. In the lytic replication cycle, expression of viral IE genes (which occurs in the absence of *de novo* protein synthesis) serves to transactivate expression of early (E) genes. Removal of essential IE genes from the HSV genome results in the creation of vectors that are unable to enter the lytic cycle in non-complementing cells, but nonetheless are transported in a normal fashion to the nucleus, where they establish a persistent latent state [27,28].

Gene transfer to muscle in the treatment of neuropathy

Transduction of muscle to achieve systemic release of the peptide has been accomplished using both non-viral and viral vectors. Injection of a plasmid coding for murine NT-3 protects against the development of neuropathy caused by a toxic dose of cisplatin [29]. In that report, the mice were given two injections of a plasmid coding for NT-3 augmented by electric pulses. Three days after the plasmid injection cisplatin administration commenced, and the plasmid injection was repeated at the 2 week time point. Injection of 5 or 50 µg of plasmid (but not 1 µg) produced a 50% improvement in the distal latency of the caudal sensory nerve of the tail, the only nerve parameter measured in that study [29]. Plasmid injection resulted in an increase in plasma NT-3 levels that declined over the course of several weeks. In a rabbit model of ischemic peripheral neuropathy, intramuscular injection of a plasmid coding for VEGF resulted in a substantial improvement in compound muscle action potential amplitude, motor nerve conduction velocity, sensory nerve amplitude, and conduction velocity in the sciatic nerve [30]. In both the STZ rat and alloxan-treated rabbit models of diabetic neuropathy, intramuscular injection of the plasmid coding for VEGF resulted in normalization of the motor and sensory nerve conduction velocity, as well as a return of thermal sensation in the tail, a function mediated by small fibers [31]. Whether the VEGF effect is mediated through angiogenesis or represents direct protective effects on neurons and Schwann cells has not been established. In a recent study, Kato and colleagues [32] reported that repeated weekly intramuscular injections of a liposome containing UV-inactivated haemagglutinating virus (Japan) and the human hepatocyte growth factor gene resulted in a significant partial correction in motor nerve conduction velocity and compound muscle action potential in the sciatic nerve when compared with the injected anterior tibial muscle 6 weeks later. There was no difference in morphometric analysis of nerve among any of the groups.

Similar results have been obtained using an adenoviral vector in place of the naked plasmid. Intramuscular injection of an adenoviral vector coding for NT-3 and defective for the adenoviral early gene functions E1 and E3, and produced a slightly greater increase in NT-3 detected in plasma than that produced by intramuscular injection of the plasmid alone, and resulted in a similar improvement in caudal nerve distal latency [33]. In the STZ model of diabetic neuropathy in the rat, intramuscular injection of the defective adenoviral vector coding for NT-3 resulted in an < 50% preservation of motor nerve conduction velocity in the sciatic nerve and 50% preservation of the caudal nerve sensory amplitude [34]. In rats intoxicated with acrylamide, intramuscular injection of the adenoviral vector coding for NT-3 produced a statistically significant but modest preservation of behavioral measures of

distal nerve function [34], and in a recent report, Sahenk and co-workers reported the use of an AAV-based vector expressing NT-3 a mouse model of hereditary sensory and motor neuropathy.

While gene transfer to muscle using either non-viral or viral-based vectors has demonstrated utility in preclinical studies using rodent models of neuropathy, there are issues inherent in scaling up from rodent to human that may make it difficult to translate the preclinical success into a human treatment. A cautionary lesson may be gleaned from the experience in gene therapy for hemophilia where impressive results in rodent and dog models of the disease achieved by injection of an AAV vector into muscle could not be translated into a human trial, because the volume of vector scaled up from the small animals to humans precluded delivery into muscle of patients. In consequence, the investigators switched to intrahepatic artery injection to transduce the liver, but this resulted in liver inflammation as the dose was increased and the project was ultimately scrapped.

Gene transfer to dorsal root ganglion for the treatment of neuropathy

We have tested non-replicating HSV vectors in several different models of neuropathy leading up to studies in diabetes. The first studies involved a selective large myelinated fiber degeneration caused by high-dose pyridoxine (PDX). This analogue of a rare human neuropathy is a useful model system for proof-of-principle studies, as the toxin has a selective neurotoxic effect on large DRG neurons with unambiguous histological, electrophysiological and correlated behavioral manifestations [35]. Subcutaneous inoculation of a non-replicating HSV vector coding for NT-3 results in transduction of lumbar DRG neurons to express NT-3 [36] and animals inoculated with the NT-3-expressing HSV vector show preservation of sensory nerve amplitude, sensory nerve conduction velocity, and amplitude of the H-wave; protection of large myelinated fiber proprioceptive sensory function, and preservation of large myelinated fibers in nerve and in the dorsal horn of spinal cord [36]. Similar results were obtained by injection of a non-replicating HSV vector expressing NGF [37]. In another model of toxin-induced neuropathy caused by the chemotherapeutic agent cisplatin, subcutaneous inoculation of HSV vectors constructed to express either NGF or NT-3 just prior to a 6-week course of cisplatin, resulted in significant preservation of sensory nerve amplitude and H-wave [38], significant protection of nerve behavioral function, and protection of the most distal branches of sensory nerve, determined by the number of protein gene product (PGP) 9.5 immunoreactive fibers in the skin [38]. Protection against degeneration was also indicated by preservation of the central terminals of small-fiber afferents in the dorsal horn of spinal cord, measured by the area of SP and CGRP immunoreactivity in the spinal cord [38].

In the model of type 1 diabetes in Swiss Webster mice created by injection with streptozotocin (STZ, 100 mg/kg 2 days apart), the mice develop diabetes characterized by elevated blood glucose and complicated by a mild neuropathy characterized by a 30% reduction in the amplitude of the sensory nerve action potential. These animals do not require insulin supplementation, and although the animals lose body weight over the course of weeks weight loss is < 15% of total body weight and the animals do not appear overtly ill. Subcutaneous inoculation of an NGF-expressing non-replicating HSV vector into both hind feet 2 weeks after the induction of diabetes prevents the loss of sensory nerve action potential amplitude characteristic of neuropathy measured 4 and 8 weeks after the injection of STZ. Preservation of small fiber function was demonstrated by the preservation of SP and CGRP RNA levels in DRG [39]. Nerve branches in the skin of the foot, measured by PGP9.5 immunostaining is similarly preserved in the animals inoculated with the NGF-expressing HSV vector.

In the same model, subcutaneous inoculation of a non-replicating HSV vector expressing vascular endothelial growth factor (VEGF) 2 weeks after the onset of diabetes prevents the reduction in foot sensory nerve amplitude characteristic of diabetes, measured 4 weeks after vector inoculation and 6 weeks after the induction of diabetes [40]. Diabetic animals demonstrate reduced thermal sensation manifested by an increased latency to withdraw from heat. Diabetic mice inoculated with the VEGF-expressing HSV vector showed preservation of pain sensation [40]. Diabetic animals also show evidence of diabetic autonomic neuropathy, measured by the number of sweat droplets formed over a 10-minute period after injection of pilocarpine. The production of sweat droplets in response to pilocarpine is substantially preserved in animals inoculated with the VEGF-expressing HSV vector [40]. Similar to the NGF-expressing HSV vector, the VEGF-expressing HSV vector preserved distal nerve branches in the skin, measured by PGP 9.5 immunostaining, and levels of CGRP and SP in the dorsal horn of spinal cord, measured by immunocytochemistry, were preserved in animals inoculated with the VEGF-expressing HSV vector [40].

Clinically significant human neuropathies are most often subacute or chronic conditions, progressing over a course of months to years; as a result of this, treatments need to be effective for a prolonged period of time. In HSV-mediated gene transfer, the duration of transgene expression *in vivo* is dependent on the promoter element employed. The native HSV latency-associated promoter 2 (LAP2) element (nucleotides 118866–119461 of the HSV genome) is the sequence responsible for lifelong expression of LATs in neurons infected with wild-type virus [41]. LAP2 was originally identified as a sequence capable of driving reporter transgene expression in transient assays *in vitro*, subsequently confirmed in DRG *in vivo* [42]. This sequence is essentially identical to the ‘long-term expression element’ that is required to prevent the shutoff of LAP1-driven reporter transgene expression during latent infection of DRG *in vivo* [43], and represents a transposable sequence capable of driving long-term transgene expression [41,42]. Others have shown that a LAP2 human cytomegalovirus fusion promoter is capable of providing prolonged expression of reporter transgenes in the nervous system *in vivo* [44–46]. The utility of LAP2-driven transgene expression has been tested using a non-replicating HSV vector with NT-3 expression under the transcriptional control of LAP2. Rats were inoculated subcutaneously in the plantar surface of both hind paws with the LAP2-driven NT-3-expressing HSV vector and expressed stable amounts of NT-3 (detected by ELISA) over a course of 6 months [47]. Five and a half months after vector inoculation, the animals were treated with PDX for 8 days in a fashion identical to that employed in the subacute experiments described earlier. As PDX was administered at five and a half months after gene transfer, protection against the development of neuropathy provided unambiguous evidence that the protection afforded by gene transfer was effective months after inoculation of the vector. As in the younger animals, 8-month-old rats treated with PDX developed neuropathy characterized by a 50% reduction in sensory amplitude and a complete loss of H-waves [47]. Animals inoculated with the LAP2-driven NT-3-expressing vector five and a half months prior to PDX intoxication showed preservation of sensory nerve amplitude and H-wave. In behavioral testing, rats transduced with the LAP2-driven NT-3-expressing vector five and a half months prior to PDX intoxication performed substantially better than PDX-only animals, both qualitatively and quantitatively [47], and had preservation of a greater number of large fibers than animals inoculated with control vector intoxicated with PDX [47].

To determine whether prolonged expression of neurotrophin-3 (NT-3) achieved by HSV-mediated gene transfer with gene expression under the control of the HSV latency promoter can provide protection against the progression of diabetic neuropathy over the course of 6 months, mice with diabetes induced by streptozotocin (STZ) were inoculated subcutaneously into both hind feet with a nonreplicating HSV vector containing the coding

sequence for neurotrophin-3 (NT-3) under the control of the HSV latency-associated promoter 2 (LAP2) element or with a control vector [48]. Animals inoculated with the NT-3 expressing vector but not animals inoculated with control vector showed preservation of sensory and motor nerve amplitude and conduction velocity measured electrophysiologically, small fiber sensory function assessed by withdrawal from heat, autonomic function measured by pilocarpine-induced sweating, skin innervation assessed by protein gene product (PGP) 9.5 staining of axons, and density of calcitonin gene-related peptide (CGRP) terminals in the spinal cord measured by immunohistochemistry 5-1/2 months after vector inoculation [48]. These results indicate that continuous production of NT-3 by LAP-2 driven expression of the transgene from an HSV vector over 6 months protects against progression of diabetic neuropathy in mice, and provide proof-of-principle demonstration for development of a novel therapy for preventing progression of diabetic neuropathy.

Summary and Implications

Gene therapy for human neuropathy is moving towards clinical application. A Phase 1/2 human trial of VEGF administered by injection of a plasmid containing the VEGF gene into muscle for treatment of diabetic neuropathy is now in progress [49]. The recent demonstration that NT-3 may be beneficial in the treatment of an inherited neuropathy suggests that this class of neuropathy may similarly be amenable to treatment by gene delivery to express neurotrophic factors [50]. However, many questions remain to be answered. Can levels of neurotrophic or neuroprotective factors adequate to preserve nerve function be achieved by intramuscular gene transfer in humans? HSV-mediated transfer to DRG neurons from the skin may not require the same scale-up in dosing as required in muscle, but will skin delivery prove effective in human neuropathy? A phase 1 trial of a non-replicating HSV vector (carrying the gene for preproenkephalin) delivered by injection into the skin to treat pain in patients with cancer has been approved by the FDA and is scheduled to start later this year. The results of that study will provide important proof of principle and safety information that will inform the future development of gene therapy for treatment of neuropathy. But prolonged expression of neurotrophic factors will probably require the development of a vector with regulatable expression in order to be applied safely to patients.

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References

1. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 1993;329:977–986. [PubMed: 8366922]
2. Brewster WJ, Fernyhough P, Diemel LT, Mohiuddin L, Tomlinson DR. Diabetic neuropathy, nerve growth factor and other neurotrophic factors. *Trends Neurosci* 1994;8:321–325. [PubMed: 7974749]
3. Anand P, Terenghi G, Warner G, Kopelman P, Williams-Chestnut RE, Sinicropi DV. The role of endogenous nerve growth factor in human diabetic neuropathy. *Nature Medicine* 1996;2:703–707.
4. Apfel SC, Arezzo JC, Brownlee M, Federoff H, Kessler JA. Nerve growth factor administration protects against experimental diabetic sensory neuropathy. *Brain Res* 1994;634:7–12. [PubMed: 7512429]

5. Diemel LT, Brewster WJ, Fernyhough P, Tomlinson DR. Expression of neuropeptides in experimental diabetes; effects of treatment with nerve growth factor or brain-derived neurotrophic factor. *Brain Res* 1994;21:171–175.
6. Mizisin AP, Bache M, DiStefano PS, Acheson A, Lindsay RM, Calcutt NA. BDNF attenuates functional and structural disorders in nerves of galactose-fed rats. *J Neuropathol Exp Neurol* 1997;56:1290–1301. [PubMed: 9413278]
7. Huang TJ, Sayers NM, Verkhatsky A, Fernyhough P. Neurotrophin-3 prevents mitochondrial dysfunction in sensory neurons of streptozotocin-diabetic rats. *Exp Neurol* 2005;194:279–283. [PubMed: 15899264]
8. Mizisin AP, Calcutt NA, Tomlinson DR, Gallagher A, Fernyhough P. Neurotrophin-3 reverses nerve conduction velocity deficits in streptozotocin-diabetic rats. *J Peripher Nerv Syst* 1999;4:211–221. [PubMed: 10642089]
9. Calcutt NA, Freshwater JD, Mizisin AP. Prevention of sensory disorders in diabetic Sprague-Dawley rats by aldose reductase inhibition or treatment with ciliary neurotrophic factor. *Diabetologia* 2004;47:718–724. [PubMed: 15298349]
10. Mizisin AP, Vu Y, Shuff M, Calcutt NA. Ciliary neurotrophic factor improves nerve conduction and ameliorates regeneration deficits in diabetic rats. *Diabetes* 2004;53:1807–1812. [PubMed: 15220205]
11. Cameron NE, Cotter MA. The neurocytokine, interleukin-6, corrects nerve dysfunction in experimental diabetes. *Exp Neurol* 2007;207:23–29. [PubMed: 17560573]
12. Christianson JA, Riekhof JT, Wright DE. Restorative effects of neurotrophin treatment on diabetes-induced cutaneous axon loss in mice. *Exp Neurol* 2003;179:188–199. [PubMed: 12618126]
13. Christianson JA, Ryals JM, McCarson KE, Wright DE. Beneficial actions of neurotrophin treatment on diabetes-induced hypoalgesia in mice. *J Pain* 2003;4:493–504. [PubMed: 14636817]
14. Calcutt NA, Allendoerfer KL, Mizisin AP, Middlemas A, Freshwater JD, Burgers M, Ranciatto R, Delcroix JD, Taylor FR, Shapiro R, et al. Therapeutic efficacy of sonic hedgehog protein in experimental diabetic neuropathy. *J Clin Invest* 2003;111:507–514. [PubMed: 12588889]
15. Brussee V, Cunningham FA, Zochodne DW. Direct insulin signaling of neurons reverses diabetic neuropathy. *Diabetes* 2004;53:1824–1830. [PubMed: 15220207]
16. Kamiya H, Zhang W, Ekberg K, Wahren J, Sima AA. C-Peptide reverses nociceptive neuropathy in type 1 diabetes. *Diabetes* 2006;55:3581–3587. [PubMed: 17130507]
17. Nakae M, Kamiya H, Naruse K, Horio N, Ito Y, Mizubayashi R, Hamada Y, Nakashima E, Akiyama N, Kobayashi Y, et al. Effects of basic fibroblast growth factor on experimental diabetic neuropathy in rats. *Diabetes* 2006;55:1470–1477. [PubMed: 16644707]
18. Bianchi R, Buyukakilli B, Brines M, Savino C, Cavaletti G, Oggioni N, Lauria G, Borgna M, Lombardi R, Cimen B, et al. Erythropoietin both protects from and reverses experimental diabetic neuropathy. *Proc Natl Acad Sci U S A* 2004;101:823–828. [PubMed: 14718663]
19. Calcutt NA, Jolivald CG, Fernyhough P. Growth factors as therapeutics for diabetic neuropathy. *Curr Drug Targets* 2008;9:47–59. [PubMed: 18220712]
20. Apfel SC, Kessler JA, Adornato BT, Litchy WJ, Sanders C, Rask CA. Recombinant human nerve growth factor in the treatment of diabetic polyneuropathy. NGF Study Group. *Neurology* 1998;51:695–702. [PubMed: 9748012]
21. Apfel SC, Schwartz S, Adornato BT, Freeman R, Biton V, Rendell M, Vinik A, Giuliani M, Stevens JC, Barbano R, et al. Efficacy and safety of recombinant human nerve growth factor in patients with diabetic polyneuropathy: A randomized controlled trial. *JAMA* 2000;284:2215–2221. [PubMed: 11056593]
22. Apfel SC, Arezzo JC, Lipson L, Kessler JA. Nerve growth factor prevents experimental cisplatin neuropathy. *Ann Neurol* 1992;31:76–80. [PubMed: 1543351]
23. Poduslo JF, Curran GL. Permeability at the blood-brain and blood-nerve barriers of the neurotrophic factors: NGF, CNTF, NT-3, BDNF. *Brain Res Mol Brain Res* 1996;36:280–286. [PubMed: 8965648]
24. Fink DJ, DeLuca NA, Goins WF, Glorioso JC. Gene transfer to neurons using herpes simplex virus-based vectors. *Annu Rev Neurosci* 1996;19:265–287. [PubMed: 8833444]

- 25. Glorioso JC, Fink DJ. Herpes vector-mediated gene transfer in treatment of diseases of the nervous system. *Annu Rev Microbiol* 2004;58:253–271. A concise summary of basic HSV virology as it relates to gene transfer to the nervous system. [PubMed: 15487938]
- 26. Roizman, B.; Sears, A. Herpes simplex viruses and their replication. In: Fields, BN.; Knipe, DM.; Howley, PM.; Chanock, RM.; Hirsch, MS.; Melnick, JL.; Monath, TP.; Roizman, B., editors. *Fields' Virology*. 3. Vol. 2. Lippincott-Raven; 1996. p. 2231-2295.
- 27. Fink DJ, DeLuca NA, Yamada M, Wolfe DP, Glorioso JC. Design and application of HSV vectors for neuroprotection. *Gene Ther* 2000;7:115–119. [PubMed: 10673716]
- 28. Wolfe D, Goins WF, Yamada M, Moriuchi S, Krisky DM, Oligino TJ, Marconi PC, Fink DJ, Glorioso JC. Engineering herpes simplex virus vectors for CNS applications. *Exp Neurol* 1999;159:34–46. [PubMed: 10486173]
- 29. Pradat PF, Finiels F, Kennel P, Naimi S, Orsini C, Delaere P, Revah F, Mallet J. Partial prevention of cisplatin-induced neuropathy by electroporation-mediated nonviral gene transfer. *Hum Gene Ther* 2001;12:367–375. [PubMed: 11242529]
- 30. Schratzberger P, Schratzberger G, Silver M, Curry C, Kearney M, Magner M, Alroy J, Adelman LS, Weinberg DH, Ropper AH, et al. Favorable effect of VEGF gene transfer on ischemic peripheral neuropathy. *Nat Med* 2000;6:405–413. [PubMed: 10742147]
- 31. Schratzberger P, Walter DH, Rittig K, Bahlmann FH, Pola R, Curry C, Silver M, Krainin JG, Weinberg DH, Ropper AH, et al. Reversal of experimental diabetic neuropathy by VEGF gene transfer. *J Clin Invest* 2001;107:1083–1092. [PubMed: 11342572]
- 32. Kato N, Nemoto K, Nakanishi K, Morishita R, Kaneda Y, Uenoyama M, Ikeda T, Fujikawa K. Nonviral gene transfer of human hepatocyte growth factor improves streptozotocin-induced diabetic neuropathy in rats. *Diabetes* 2005;54:846–854. [PubMed: 15734864]
- 33. Pradat PF, Kennel P, Naimi-Sadaoui S, Finiels F, Scherman D, Orsini C, Delaere P, Mallet J, Revah F. Viral and non-viral gene therapy partially prevents experimental cisplatin-induced neuropathy. *Gene Ther* 2002;9:1333–1337. [PubMed: 12224017]
- 34. Pradat PF, Kennel P, Naimi-Sadaoui S, Finiels F, Orsini C, Revah F, Delaere P, Mallet J. Continuous delivery of neurotrophin 3 by gene therapy has a neuroprotective effect in experimental models of diabetic and acrylamide neuropathies. *Hum Gene Ther* 2001;12:2237–2249. Report of gene transfer to muscle in the treatment of diabetic neuropathy. [PubMed: 11779407]
- 35. Helgren ME, Cliffer KD, Torrento K, Cavnor C, Curtis R, DiStefano PS, Wiegand SJ, Lindsay RM. Neurotrophin-3 administration attenuates deficits of pyridoxine-induced large-fiber sensory neuropathy. *J Neurosci* 1997;17:372–382. [PubMed: 8987762]
- 36. Chattopadhyay M, Wolfe D, Huang S, Goss J, Glorioso JC, Mata M, Fink DJ. In vivo gene therapy for pyridoxine-induced neuropathy by herpes simplex virus-mediated gene transfer of neurotrophin-3. *Ann Neurol* 2002;51:19–27. [PubMed: 11782980]
- 37. Chattopadhyay M, Goss J, Lacomis D, Goins W, Glorioso JC, Mata M, Fink DJ. Protective effect of HSV-mediated gene transfer of nerve growth factor in pyridoxine neuropathy demonstrates functional activity of trkA receptors in large sensory neurons of adult animals. *Eur J Neurosci* 2003;17:732–740. [PubMed: 12603263]
- 38. Chattopadhyay M, Goss J, Wolfe D, Goins WC, Huang S, Glorioso JC, Mata M, Fink DJ. Protective effect of herpes simplex virus-mediated neurotrophin gene transfer in cisplatin neuropathy. *Brain* 2004;127:929–939. [PubMed: 14749293]
- 39. Goss JR, Goins WF, Lacomis D, Mata M, Glorioso JC, Fink DJ. Herpes simplex-mediated gene transfer of nerve growth factor protects against peripheral neuropathy in streptozotocin-induced diabetes in the mouse. *Diabetes* 2002;51:2227–2232. The first report of HSV-mediated gene transfer in the treatment of diabetic neuropathy. [PubMed: 12086954]
- 40. Chattopadhyay M, Krisky D, Wolfe D, Glorioso JC, Mata M, Fink DJ. HSV-mediated gene transfer of vascular endothelial growth factor to dorsal root ganglia prevents diabetic neuropathy. *Gene Ther* 2005;12:1377–1384. Report demonstrating the HSV-mediated expression of VEGF is also effective in treating diabetic neuropathy. [PubMed: 15843809]

41. Goins WF, Sternberg LR, Croen KD, Krause PR, Hendricks RL, Fink DJ, Straus SE, Levine M, Glorioso JC. A novel latency-active promoter is contained within the herpes simplex virus type 1 UL flanking repeats. *J Virol* 1994;68:2239–2252. [PubMed: 8139009]
42. Goins WF, Lee KA, Cavalcoli JD, O'Malley ME, DeKosky ST, Fink DJ, Glorioso JC. Herpes simplex virus type 1 vector-mediated expression of nerve growth factor protects dorsal root ganglion neurons from peroxide toxicity. *J Virol* 1999;73:519–532. [PubMed: 9847358]
43. Lokensgard JR, Berthomme H, Feldman LT. The latency-associated promoter of herpes simplex virus type 1 requires a region downstream of the transcription start site for long-term expression during latency. *J Virol* 1997;71:6714–6719. [PubMed: 9261395]
44. Palmer JA, Branston RH, Lilley CE, Robinson MJ, Groutsi F, Smith J, Latchman DS, Coffin RS. Development and optimization of herpes simplex virus vectors for multiple long-term gene delivery to the peripheral nervous system. *J Virol* 2000;74:5604–5618. [PubMed: 10823868]
45. Lilley CE, Groutsi F, Han Z, Palmer JA, Anderson PN, Latchman DS, Coffin RS. Multiple immediate-early gene-deficient herpes simplex virus vectors allowing efficient gene delivery to neurons in culture and widespread gene delivery to the central nervous system in vivo. *J Virol* 2001;75:4343–4356. [PubMed: 11287583]
46. Perez MC, Hunt SP, Coffin RS, Palmer JA. Comparative analysis of genomic HSV vectors for gene delivery to motor neurons following peripheral inoculation in vivo. *Gene Ther* 2004;11:1023–1032. [PubMed: 15164091]
47. Chattopadhyay M, Wolfe D, Mata M, Huang S, Glorioso JC, Fink DJ. Long-term neuroprotection achieved with latency-associated promoter-driven herpes simplex virus gene transfer to the peripheral nervous system. *Mol Ther* 2005;12:307–313. [PubMed: 15927533]
- 48. Chattopadhyay M, Mata M, Goss J, Wolfe D, Huang S, Glorioso JC, Fink DJ. Prolonged preservation of nerve function in diabetic neuropathy in mice by herpes simplex virus-mediated gene transfer. *Diabetologia* 2007;50:1550–1558. Report describing the use of the latency promoter in an HSV vector to achieve long-term protection against the development of diabetic neuropathy. [PubMed: 17508196]
49. Isner JM, Ropper A, Hirst K. VEGF gene transfer for diabetic neuropathy. *Hum Gene Ther* 2001;12:1593–1594. [PubMed: 11529248]
50. Sahenk Z, Nagaraja HN, McCracken BS, King WM, Freimer ML, Cedarbaum JM, Mendell JR. NT-3 promotes nerve regeneration and sensory improvement in CMT1A mouse models and in patients. *Neurology* 2005;65:681–689. [PubMed: 16157899]