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GENE THERAPY APPROACHES TO ENHANCING PLASTICITY AND REGENERATION AFTER SPINAL CORD INJURY

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Abstract

During the past decades, new insights into mechanisms that limit plasticity and functional recovery after spinal cord injury have spurred the development of novel approaches to enhance axonal regeneration and rearrangement of spared circuitry. Gene therapy may provide one means to address mechanisms that underlie the insufficient regenerative response of injured neurons and can also be used to identify factors important for axonal growth. Several genetic approaches aimed to modulate the environment of injured axons, for example by localized expression of growth factors, to enhance axonal sprouting and regeneration and to guide regenerating axons towards their target have been described. In addition, genetic modification of injured neurons via intraparenchymal injection, or via retrograde transport of viral vectors has been used to manipulate the intrinsic growth capacity of injured neurons. In this review we will summarize some of the progress and limitations of cell transplantation and gene therapy to enhance axonal bridging and regeneration across a lesion site, and to maximize the function, collateral sprouting and connectivity of spared axonal systems.

INTRODUCTION

Regeneration and structural plasticity are regulated by a delicate balance of growth-promoting and growth-inhibiting influences in the injured adult mammalian central nervous system (CNS). Factors intrinsic to injured neurons as well as extrinsic influences on injured neurons and their axons, contribute to the lack of spontaneous regeneration. Among the extrinsic factors, a lack of neurotrophic factor support (Tuszynski and Lu, 2008, Widenfalk, et al., 2001), inhibitory molecules at the lesion site including ephrins, semaphorins, netrins and others (Benson, et al., 2005, Bolsover, et al., 2008, Low, et al., 2008, Niclou, et al., 2003), the formation of a glial scar (Fitch and Silver, 2008, Silver and Miller, 2004), inflammatory responses (Bethea and Dietrich, 2002, Popovich and McTigue, 2009), the presence of inhibitory extracellular matrix molecules such as chondroitinsulfate-proteoglycans (Silver and Miller, 2004), and myelin-based inhibitors including MAG, Omgp and NOGO (Xie and Zheng, 2008) severely limit spontaneous regeneration. Despite this abundance of inhibitory influences, some spontaneous recovery can occur after spinal cord injury (SCI). The underlying structural changes, mostly collateral sprouting, have been

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identified in some instances (Ballermann and Fouad, 2006, Kerr, 1975, Raisman and Field, 1973, Wang, et al., 1991, Weidner, et al., 2001, Z'Graggen, et al., 2000). These spontaneous changes might be one target to enhance functional recovery. However, such approaches are limited by the neuronal and axonal substrate remaining after injury, constraining the behavioral repertoire that can be supported by a limited number of spared connections. Only de novo regeneration of injured axons might be able to overcome these limitations. Regeneration requires axons to bridge across a spinal cord lesion site, to potentially grow over long distances, to reach appropriate target neurons, to reestablish synapses and to be remyelinated. In addition to these challenges, newly formed connections need to be integrated in the remaining circuitry. Given the complexity of inhibitory influences and the limited intrinsic regenerative capacity of injured neurons, it seems likely that combinatory approaches influencing intrinsic and extrinsic mechanisms need to be addressed to achieve axonal growth sufficient for robust functional recovery after spinal cord injury. Gene therapy might be one means to accomplish some of these goals (Fig. 1).

In this article we will review current approaches and discuss potential future perspectives to enhance axonal regeneration and plasticity, using genetically modified cells and in vivo gene therapy. We will focus on growth factors and regenerative programs intrinsic to injured neurons to enhance plasticity and regeneration, and discuss the limitations of current approaches.

GENETICALLY MODIFIED CELLS FOR AXONAL REGENERATION

The lack of suitable growth substrates, the inhibitory environment around a spinal cord lesion site and cystic cavities present at a lesion site, have made cell transplantation an appealing approach to overcome an otherwise impermeable barrier for regenerating axons. Early transplantation studies used primarily fetal tissue (Bregman, et al., 1993, Mori, et al., 1997, Stokes and Reier, 1992, Tessler, 1991, Tessler, et al., 1997), and peripheral nerves (David and Aguayo, 1981, Richardson, et al., 1980) to bridge a lesion site and were followed by grafts of better characterized and more homogenous cell populations including Schwann cells (Li and Raisman, 1994, Tuszynski, et al., 1998, Weidner, et al., 1999, Xu, et al., 1997, Xu, et al., 1995, Xu, et al., 1994), olfactory ensheathing cells (Li, et al., 1997, Lu, et al., 2006, Ramon-Cueto, et al., 2000, Ramon-Cueto and Nieto-Sampedro, 1994, Richter and Roskams, 2008), bone marrow stromal cells (Ankeny, et al., 2004, Hofstetter, et al., 2002) and neural stem cells (Lepore and Fischer, 2005, Lu, et al., 2003, Pfeifer, et al., 2004). Depending on the cell type, cellular grafts can fill a lesion site and thereby provide a potential substrate for axonal extension.

While cellular grafts can serve as a matrix for axonal growth into and possibly beyond sites of SCI, and in some cases induce significant growth into the lesion site, axons rarely exit the lesion site and responses vary between different axonal populations. To further enhance axonal growth additional stimuli are needed. Due to their important role in axonal growth and survival in the developing nervous system, the potential beneficial effects of neurotrophic factors have been subject to extensive investigations in the injured spinal cord, in particular in combination with cellular grafts. Since systemic administration of trophic factors can lead to adverse effects such as pain, weight loss, and Schwann cell hypertrophy and due to the inability of these polar molecules to cross the blood brain barrier, other means for the localized, targeted delivery of growth factors are needed. Autologous or syngeneic cells genetically modified by viral vectors (ex vivo gene therapy) can serve as biological minipumps for the localized, targeted delivery of growth factors into a lesion site and provide a potential substrate for axonal extension. Axonal growth in response to fibroblasts (Blesch and Tuszynski, 2003, Blesch, et al., 1999, Blesch, et al., 2004, Grill, et al., 1997, Grill, et al., 1997, Himes, et al., 2001, Hollis, et al., 2009, Jin, et al., 2002, Jin, et al., 2000, Liu, et al., 1999, Lu, et al., 2001, McTigue, et al., 1998, Tuszynski, et al., 1996, Tuszynski,

et al., 2003, Tuszynski, et al., 1994), Schwann cells (Menei, et al., 1998, Weidner, et al., 1999), bone marrow stromal cells (Lu, et al., 2005, Lu, et al., 2007a), olfactory ensheathing cells (Cao, et al., 2004, Ruitenberg, et al., 2005, Ruitenberg, et al., 2003) and neural stem/progenitor cells (Cao, et al., 2005, Liu, et al., 1999) transduced to express growth factors has been investigated. These studies clearly demonstrate that injured axons remain responsive to neurotrophic factor delivery, even in chronic stages of injury responding with enhanced neuronal survival and axon growth.

Growth factors investigated to date include members of the neurotrophin family (NGF, BDNF, NT-3, NT-4/5), neuropoietic cytokines (LIF, IL-6, CNTF), GDNF family ligands and insulin-like growth factors (IGF). Depending on the means of delivery, the types and amount of growth factors provided and the level of spinal injury, different populations of axons have been found to respond to varying degrees to trophic factor delivery. Axonal populations such as rubrospinal, raphespinal, cerulospinal, reticulospinal and other brainstem projections, as well as spinal projections including propriospinal, spinal motor axons and primary sensory axons have been shown to robustly penetrate growth factor-producing cellular grafts (Bamber, et al., 2001, Blesch and Tuszynski, 2007, Blesch, et al., 2004, Jin, et al., 2002, Jin, et al., 2000, Liu, et al., 1999, Lu, et al., 2001, Lu, et al., 2005, McTigue, et al., 1998, Menei, et al., 1998, Mitsui, et al., 2005, Tobias, et al., 2003, Ye and Houle, 1997). A notable exception is the corticospinal tract (CST), the most important projection for fine motor control in primates. Indeed, no reproducible study has clearly demonstrated growth of this axonal population for significant distances into or even across a lesion site in the spinal cord. Instead, corticospinal axons sprout around the lesion after NT-3 protein infusion (Schnell, et al., 1994), cellular NT-3 delivery (Grill, et al., 1997, Tuszynski, et al., 2003), AAV-mediated NT-3 gene delivery (Fortun, et al., 2009), and adenoviral NT-3 gene transfer in combination with peripheral nerve grafts (Blits, et al., 2000). After unilateral lesions, corticospinal axons form collaterals extending from the uninjured side towards NT-3-expressing motor neurons in the denervated contralateral spinal cord (Chen, et al., 2006, Zhou, et al., 2003). While CST axons respond to NT-3 after injury and during neural development (Schnell, et al., 1994), other trophic factors fail to promote axonal regeneration in the injured adult spinal cord, despite their role in neural development. IGF-I has essential roles in CST development (Ozdinler and Macklis, 2006) and supports corticospinal neuron survival post-injury, but fails to promote axonal regeneration in the adult injured spinal cord (Hollis, et al., 2009).

Studies investigating the influence of BDNF gene delivery on corticospinal neurons and their axons have demonstrated strong effects on neuronal survival and atrophy, but failed to show effects on axonal growth and regeneration. While BDNF injections or cellular BDNF delivery close to the cell soma in the cortex enhance the survival of CST neurons after subcortical lesion (Giehl and Tetzlaff, 1996, Lu, et al., 2001), cellular BDNF delivery in the lesioned spinal cord fails to enhance CST regeneration or sprouting (Brock, et al., 2010, Lu, et al., 2001). Interestingly, cellular BDNF delivery to the injured spinal cord has remote effects on axotomy-induced corticospinal neuronal atrophy in rodents and non-human primates (Brock, et al., 2010). BDNF can therefore act over extended distances to ameliorate neuronal degeneration. In contrast, cellular NT-3 at a cervical lesion site fails to protect corticospinal neurons from lesion-induced atrophy (Brock, et al., 2010) despite its effects on corticospinal axon sprouting (Blits, et al., 2000, Chen, et al., 2006, Grill, et al., 1997, Schnell, et al., 1994, Tuszynski, et al., 2003, Zhou, et al., 2003). These distinct effects are most likely due to differences in trkB and trkC receptor signaling or retrograde transport of activated receptor complexes and their downstream signals from the axonal compartment to the neuronal cell soma.

On the other hand, rubrospinal axonal growth and regeneration is influenced by viral and cellular BDNF delivery to the cell soma and the axonal compartment. Fibroblast and olfactory ensheathing cells genetically modified to express BDNF augment rubrospinal axon growth when grafted to a spinal cord lesion site (Liu, et al., 1999, Ruitenberg, et al., 2003). Viral BDNF gene delivery in the red nucleus prevents not only rubrospinal neuronal degeneration (Kwon, et al., 2007, Ruitenberg, et al., 2004) similar to BDNF infusions into the red nucleus (Kobayashi, et al., 1997, Kwon, et al., 2002, Plunet, et al., 2002) but also enhances axon growth into a peripheral nerve graft (Kwon, et al., 2002, Plunet, et al., 2002). Thus, influences of BDNF on rubrospinal neurons and their axons appear to be independent of the site of delivery.

SPATIAL AND TEMPORAL REGULATION OF GROWTH FACTOR EXPRESSION

The majority of studies investigating cellular growth factor delivery after spinal cord injury used viral vectors with constitutively active promoters resulting in persistent expression of neurotrophic factors within a lesion site. The continuous supply of growth factors within a lesion site and the lack of additional growth stimuli beyond sites of SCI might contribute to the lack of axonal growth across a lesion site. A continuous supply of trophic factors in the lesion site might prevent axons from extending from a growth-supportive environment within a graft into the inhospitable milieu beyond the lesion site. In addition, long-term delivery of growth factors could lead to adverse effects. Indeed, continuous expression of GDNF or NT-4/5 by genetically modified cells has been shown to result in a gradual increase in graft size due to the invasion and proliferation of Schwann cells (Blesch and Tuszynski, 2003, Blesch, et al., 2004). Thus, the ability to turn gene expression off after axons have entered a lesion site might enhance the efficacy and safety of growth factor gene delivery.

Using a tetracycline regulated BDNF expression system (Gossen and Bujard, 1992, Gossen, et al., 1995), recent studies have indicated that transient neurotrophic factor expression within a lesion site is sufficient to induce axonal growth, but is not required to sustain regenerated axons within a site of spinal cord injury (Blesch and Tuszynski, 2007). Axons extend into a lesion site in response to BDNF expressed by primary fibroblast grafts, but axons do not withdraw when BDNF expression is subsequently turned off. Thus high levels of trophic factors are needed to initiate axonal growth, but not to sustain axons that have regenerated.

Therapeutic approaches would clearly benefit from novel vectors that allow for transient gene delivery, such as promoters that automatically shut off after several weeks to months, or promoters that can respond to a changing environment by turning gene expression off once cells grafted to a lesion site differentiate. An alternative approach to regulating the expression of growth factors might be to regulate growth factor receptor signal transduction. A neurotrophin receptor with regulated kinase activity, that can be activated by a small ligand would limit downstream receptor signaling to a defined time period and might be another means of temporally stimulating cell-intrinsic regenerative programs. Recent studies have demonstrated the feasibility of this approach (Alfa, et al., 2009). Lentiviral vectors expressing chimeric trk receptors that lack the extracellular domain for ligand binding, but contain an intracellular domain for synthetic small molecule-induced dimerization, can regulate axonal growth in vitro. Thus, pharmacological modulation of neurotrophin activity can be accomplished.

Although regulated gene transfer in a lesion site might be one step to enhance axonal bridging across a lesion site, turning off growth factor gene expression in the lesion site without additional growth stimuli beyond the lesion site is insufficient for axons to extend into the distal spinal cord. If adult injured axons respond similarly as axons in the

developing nervous system, growth factor gradients might help to establish the topography of target innervation by regenerating axons (Ma, et al., 2002, Markus, et al., 2002, Marotte, et al., 2004, Tessier-Lavigne and Goodman, 1996, Tucker, et al., 2001). Concentration gradients of trophic factors distal to the lesion site could presumably promote chemotropic directional axon growth beyond a lesion site and even into an appropriate target. Neurotrophin gradients can be established by viral gene transfer and recent studies have demonstrated that transplants of postnatal dorsal root ganglion (DRG) neurons can extend axons along neurotrophin gradients in adult white matter (Jin, et al., 2008, Ziemba, et al., 2008).

In the injured spinal cord, gradients of NT-3 have been shown to promote axonal bridging across a cervical lesion site. Injection of lentiviral vectors expressing NT-3 in the dorsal columns rostral to a lesion establishes a gradient of NT-3 with highest levels beyond the lesion. Ascending sensory axons shown to respond to NT-3 (Bradbury, et al., 1999) extend not only into a lesion filled with bone marrow stromal cells, but also across the cellular graft and for short distances into the distal host spinal cord. (Taylor, et al., 2006). Using a similar approach, viral NT-3 expression in the nucleus gracilis, the target of ascending sensory axons, has been shown to promote axonal bridging across the lesion site and reinnervation of the original target (Alto, et al., 2009). However, if NT-3-expressing virus is mistargeted and expressed in a different region, axons fail to reinnervate the nucleus gracilis and instead grow towards an inappropriate target where the highest concentration of NT-3 can be found.

Targeted expression of FGF-2 and NGF by adenoviral gene transfer to the dorsal spinal cord can also induce directed sensory axon regrowth across the dorsal root entry zone into the spinal cord (Romero, et al., 2001). Combining NGF gene transfer in the superficial layers of the spinal cord with semaphorin 3A gene transfer to deeper layers further restricts sensory axon regeneration to appropriate targets (Tang, et al., 2007, Tang, et al., 2004). Thus, adult regenerating axons are not only responsive to attractive but also to repulsive guidance cues.

Gradients of neurotrophins established by lentiviral BDNF gene transfer have also been shown to induce axonal sprouting from neural restricted precursors towards the highest concentration of BDNF (Bonner, et al., 2010). These grafts could serve as cellular relays across the lesion site if transplanted neurons receive appropriate synaptic input and are able to form appropriate connections with downstream targets. Similarly, descending reticulospinal axons can bridge across a lesion site if high BDNF levels are expressed caudal to a C5 hemisection lesion (Lu, et al., 2007b). These results will for the first time permit a detailed examination of the contribution of unmistakably regenerated axons to functional recovery after spinal cord injury.

GENE TRANSFER TO ACTIVATE NEURON-INTRINSIC GROWTH PROGRAMS

The failure to activate intrinsic regenerative programs is likely to contribute to the limited axonal regeneration in the injured spinal cord. An insufficient upregulation of transcriptional programs may underlie the inadequate expression of growth-associated proteins (Fernandes, et al., 1999, Goldberg, et al., 2002, Stam, et al., 2007, Xiao, et al., 2002), ineffective protein synthesis (Park, et al., 2008) and intracellular signal activation (Filbin, 2003).

Genes and proteins that support axonal outgrowth during neuronal development, and regeneration in the adult peripheral nervous system have provided some insight into processes that might be able to activate the intrinsic capacity to regenerate in the injured adult CNS. One means to stimulate central axon regeneration of dorsal root ganglion neurons is the application of “conditioning lesions” to the peripheral branch of sensory neurons. Lesions in the peripheral nervous system lead to rapid and sustained changes in gene expression (Costigan, et al., 2002, Goldberg, et al., 2002, Michaelievski, et al., 2010,

Stam, et al., 2007, Xiao, et al., 2002) and at least some of these transcriptional changes contribute to the intrinsic ability of sensory neurons to regenerate their axons (Smith and Skene, 1997). Findings indicating that these genetic programs retain their growth promoting effects even in chronic spinal cord injury (Kadoya, et al., 2009) support the notion that these regulatory networks are of clinical relevance. Several signaling pathways that contribute to the regenerative capacity of DRG neurons “conditioned” by lesions in the peripheral axon branch have been identified. Among those are upregulation of cAMP signaling pathways (Cai, et al., 2001, Lu, et al., 2004, Neumann, et al., 2002, Qiu, et al., 2002), cytokines (Cafferty, et al., 2004, Cao, et al., 2006, Qiu, et al., 2005, Wu, et al., 2006) and trophic factors (Gao, et al., 2003). Indeed, gene transfer of transcription factors such as CREB (cAMP response element binding protein) (Gao, et al., 2004), ATF3 (activating transcription factor 3) (Seijffers, et al., 2006, Seijffers, et al., 2007) or STAT3 (signal transducer and activator of transcription 3) (Qiu, et al., 2005) has been shown to enhance axonal growth but in vivo effects are limited to short-distance sprouting around the lesion site. Additional studies are needed to better understand the intracellular pathways regulating the regenerative capacity of injured neurons and concurrent initiation of several transcriptional programs might be needed to fully activate signaling cascades for axonal regeneration. Activating the intrinsic growth capacity in combination with growth stimulation at the lesion site is likely to further enhance axonal regeneration. Recent studies on sensory axon regeneration support this view (Alto, et al., 2009, Kadoya, et al., 2009, Lu, et al., 2004).

Neuronal overexpression of proteins playing a major role in CNS development, like retinoic acid receptor beta (RAR β) (Wong, et al., 2006, Yip, et al., 2006) and neuronal calcium sensor-1 (NCS1) (Yip, et al., 2010), or Schwann cells expressing polysialyltransferase to increase PSA-NCAM expression (Zhang, et al., 2007) have also shown promise in augmenting the growth of injured axons. Lentiviral overexpression of the transcription factor RAR β 2 in DRG neurons enables the regeneration of injured sensory axons across the inhibitory dorsal root entry zone and improves functional recovery in sensorimotor behavioral tasks. The mechanisms underlying the augmented growth are not entirely clear, but appear to be at least partially mediated by increases in cAMP levels. Increased PI3K/Akt signaling by viral overexpression of NCS-1 in corticospinal neurons, induces collateral sprouting of uninjured axons and short distance regeneration of injured axons in the spared gray matter (Yip, et al., 2010). The latter study used lentiviral vectors expressing the gene of interest together with GFP to specifically label axons of transduced neurons. A similar approach for the rapid cloning and identification of genes with growth-promoting properties has also been described (Low, et al., 2010). These tools will be valuable in future studies to identify additional genes with axonal growth-promoting properties. Genes that are developmentally downregulated and promote neurite growth might also provide targets for future gene transfer studies to injured neurons (Blackmore, et al., 2010, Moore, et al., 2009).

Activating neurotrophin signaling in injured neurons, by viral BDNF gene delivery or protein infusions to rubrospinal and corticospinal neurons, can also increase expression of regeneration-associated genes such as GAP-43 and β III-tubulin (Kobayashi, et al., 1997, Kwon, et al., 2007, Ruitenberg, et al., 2004), thereby enhancing axonal sprouting (Kobayashi, et al., 1997, Kwon, et al., 2002, Vavrek, et al., 2006). However, the activation of intrinsic receptors for BDNF is insufficient to induce corticospinal axon growth into a BDNF-expressing graft. Only if intrinsic neuronal growth mechanisms are enhanced by overexpression of trkB in adult corticospinal motor neurons, can corticospinal axons regenerate into cellular grafts expressing BDNF (Hollis, et al., 2009). While these studies demonstrate for the first time regeneration of injured corticospinal axons into a cellular graft, trkB trafficking was limited to subcortical axons, and only if BDNF-expressing grafts were placed into a subcortical lesion site, axon growth was observed. In contrast, BDNF delivery in the spinal cord failed to induce axon regeneration, most likely due to the lack of

trkB transport to more distal spinal cord axons. Eliciting corticospinal regeneration into spinal cord lesions will therefore require a means to enhance trkB trafficking along injured axons.

TARGETED GENE DELIVERY

The studies described above have clearly shown that ex vivo (cellular) gene transfer and direct injection of viral vectors (in vivo gene transfer) are potent means to promote plasticity and axonal regeneration. Advances in viral vectors have made these approaches possible and additional developments in vector administration and design might further enhance the specificity and feasibility of viral gene transfer.

The need for reliable means to regulate gene expression or to limit the duration of gene expression has been discussed before. Novel ways of vector delivery could also enhance the practicability of therapeutic gene delivery and reduce the invasiveness of intraparenchymal injections. In particular, the ability of viral vectors to be retrogradely transported along an axon from the injection site is of high interest in the spinal cord. Adenovirus, herpes virus, adeno-associated virus (AAV) and lentivirus injected in skin, muscle or peripheral nerves, can be taken up by nerve terminals and axons and transported to motor neurons in the spinal cord and sensory neurons in the dorsal root ganglia. (Azzouz, et al., 2004, Boulis, et al., 2003, Fortun, et al., 2009, Ghadge, et al., 1995, Glatzel, et al., 2000, Haase, et al., 1997, Kaspar, et al., 2003, Keir, et al., 1995, Kelkar, et al., 2006, Wong, et al., 2004).

One of the best-characterized and most versatile vector systems for CNS gene delivery and retrograde transport is AAV. At least 12 different serotypes (AAV1-12), and more than 100 AAV variants have been described (Grimm and Kay, 2003, Wu, et al., 2006) that differ tremendously in their affinity to cellular receptors. These differences explain the variability in cell-specific infectivity in the CNS (Blits, et al., 2010, Burger, et al., 2004, Mason, et al., 2010, Taymans, et al., 2007) and differences in their retrograde transport capabilities (Hollis, et al., 2008). Comparisons of different AAV serotypes have shown that AAV 1, 4 and 6 have higher retrograde transport efficiency to motor neurons than other serotypes tested to date (Hollis, et al., 2008, Towne, et al., 2010). AAV 1, 5, 6 appear to be more efficient in transducing sensory neurons after direct DRG injection (Mason, et al., 2010).

Despite promising results after retrograde neuronal transduction in the spinal cord, the efficacy of retrograde AAV gene transfer can be further improved. The majority of virus-particles remain in the vicinity of the injection site, and only a small proportion of particles are retrogradely transported. Different approaches have been taken to improve the retrograde transport of AAV and gene expression. Double-stranded AAV variants (scAAV, self-complementary AAV) (McCarty, et al., 2003, McCarty, et al., 2001) are about 20 times more effective in retrograde motor neuron infection after intramuscular administration, compared to standard single-stranded AAV (ssAAV) (Hollis, et al., 2008). Self-complementary AAV are also superior in an alternative delivery approach with high specificity for DRG neurons: intrathecal injection of AAV into the cerebrospinal fluid. AAV1 appears to be superior to AAV5 in transducing DRG neurons (Storek, et al., 2006) and the transduction efficacy of AAV8 has been reported to be even higher (Storek, et al., 2008). The improvements in retrograde infection with scAAV compared to single-stranded AAV vectors are most likely not a result of improved retrograde virus transport but due to the elimination of the rate-limiting step of second-strand DNA synthesis necessary for successful AAV transduction. A disadvantage of this strategy is the loss of about 50 % of the packing-capacity for transgene expression (2,5 kb). Demyelination of peripheral nerves also improves the retrograde transport of AAV and adenovirus (Hollis, et al., 2010, Zhang, et al., 2010). Lastly, targeted evolution of the AAV-capsid has the potential to considerably improve retrograde AAV transport and cell-tropism, warranting further investigations (Gray,

et al., 2010, Wu, et al., 2006). Retrograde viral transport together with cell-specific promoters might allow for cell specific gene expression in motor neurons to chemotropically attract descending fibers, and to enhance regenerative programs in ascending sensory fibers or in other neuronal populations that are difficult to target such as corticospinal neurons.

CONCLUSIONS AND FUTURE DIRECTIONS

Gene therapy for neurological disorders has moved from preclinical studies to several Phase I and II clinical trials in neurodegenerative diseases including Alzheimer's disease and Parkinson's disease (Christine, et al., 2009, Kaplitt, et al., 2007, Mandel, 2010, Marks, et al., 2008, Tuszynski, et al., 2005). Whereas chronic neurodegenerative diseases might require long-term gene delivery to prevent progressive neuronal degeneration or to change a neuronal phenotype, gene therapeutic approaches in spinal cord injury will likely require systems for the transient expression of therapeutic genes in injured neurons and their environment. The translation of animal studies will therefore advance with improved vector and promoter systems for minimally invasive administration and targeted, localized, cell-specific and regulated gene delivery. A combination of approaches that address barriers for axonal regeneration at the site of injury, such as suitable substrates for axonal growth, molecules for axonal guidance, and manipulations of the intrinsic signaling cascade to stimulate the regenerative capacity of injured neurons may be needed to maximize recovery of function. Gene transfer to supply factors stimulating axonal growth within and beyond the lesion site and to activate the neuron-intrinsic capacity for axonal regeneration might play an integral part of such combinatorial approaches.

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Figure 1.
Schematic outline of potential gene therapy approaches for regeneration in the injured spinal cord.

Figure 2. A combination of conditioning lesion and NT-3 gene transfer allows ascending dorsal column sensory axon regeneration across a spinal cord lesion site

(A). Dorsal column sensory axon transganglionically traced with cholera toxin beta extend into a lesion site filled with bone marrow stromal cells and beyond the lesion site towards a gradient of NT-3 established by lentiviral NT-3 gene transfer. (B) Higher magnification of boxed area in (A) shows numerous regenerating axons (arrowheads) that have bridged the lesion site. (C) In contrast, few axons extend into the lesion site and do not cross in the host spinal cord after a conditioning lesion without NT-3 gene delivery. Lesion site in (A) and (C) is outlined by dashed lines. Rostral is to the left, dorsal to the top. Scale bar = 250 μm in (A), 25 μm in (B), 100 μm in (C).