PERSPECTIVES

# Gene therapy and the regeneration of retinal ganglion cell axons

Because the adult mammalian central nervous system (CNS) has only limited intrinsic capacity to regenerate connections after injury, due to factors both intrinsic and extrinsic to the mature neuron (Shen et al., 1999; Berry et al., 2008; Lingor et al., 2008; Sun and He, 2010; Moore et al., 2011), therapies are required to support the survival of injured neurons and to promote the long-distance regrowth of axons back to their original target structures. The retina and optic nerve (ON) are part of the CNS and this system is much used in experiments designed to test new ways of promoting regeneration after injury (Harvey et al., 2006; Benowitz and Yin, 2008; Berry et al., 2008; Fischer and Leibinger, 2012). Testing of therapies designed to improve retinal ganglion cell (RGC) viability also has direct clinical relevance because there is loss of these centrally projecting neurons in many ophthalmic diseases.

Many different approaches are being trialled, targeting different receptor systems and/or different signalling pathways, some aimed at enhancing intrinsic growth capacity in injured RGCs, others aimed at reducing the impact of factors external to the neuron that suppress/restrict the regenerative response. An approach of interest increasingly involves the use of modified, replication-deficient viral vectors to introduce appropriate genes into injured cells in the visual pathway (gene therapy). In this brief review, I summarize recent gene therapy research from our laboratory, using the rodent visual system as an experimental model, which is aimed at improving both the viability and regenerative capacity of injured adult RGCs.

Some initial gene therapy studies used the adenoviral vector to package genes encoding various neurotrophic factors such as ciliary neurotrophic factor (CNTF), brain-derived neurotrophic factor (BDNF) or glial cell-line derived neurotrophic factor (GDNF) (Di Polo et al., 1998; Weise et al., 2000; Schmeer et al., 2002; van Adel et al., 2005). More recently, the vector of choice to obtain more selective transduction of RGCs is the adeno-associated virus (AAV). The vectors are injected into the posterior (vitreal) chamber of the eye to target RGCs in inner retina. AAV vectors are non-enveloped single-stranded DNA viruses and show minimal pathogenicity or toxicity. Indeed, clinical trials involving the sub-retinal injection of these recombinant, replication-deficient vectors to target cells in outer retinal margins have begun (Bainbridge et al., 2008; Simonelli et al., 2010).

Many different AAV serotypes exist, but the most widely used for RGC transduction is AAV2 (Martin et al., 2003; Sapieha et al., 2003; Fischer et al., 2004; Zhou et al., 2005; Leaver et al., 2006a,b; Hellström et al., 2009; Pease et al., 2009; Kurimoto et al., 2010; Park et al., 2010; Hellström et al., 2011a,b). This vector provides long-term transduction of adult RGCs – we have seen maintained transduction and expression of transgenes 15 months after the initial intraocular injection (Hellström and Harvey 2011). Similar to many groups we use bi-cistronic AAV2 vectors that encode a gene of interest as well as a marker gene such as green fluorescent protein (GFP). This permits identification of the transduced RGC population in injected eyes, and also allows visualization of axons from transduced RGCs in the optic nerve and brain (Harvey et al., 2002; Leaver et al., 2006a). In our studies, we have examined the effects of various AAV vectors in adult rodent models after optic nerve (ON) crush or, more commonly, after transplantation of a segment of autologous peripheral nerve (PN) onto the cut ON, a procedure that permits injured RGCs to regenerate axons for longer distances (Heiduschka and Thanos, 2000; Harvey et al., 2006; Watanabe 2010). This graft method also allows us to apply retrograde tracers to the distal end of PN grafts and thus we can assess not only RGC viability and morphology (by immunostaining with antibodies to β-III tubulin) but also quantify the proportion of surviving RGCs able to regenerate an axon – an approach that has proved especially fruitful and enlightening. By inserting such PN grafts into central visual target regions it is possible to test true regenerative responses physiologically and behaviourally.

Our initial ON crush study examined the impact of three different AAVs containing genes encoding either a secretable form of CNTF, BDNF or growth-associated protein-43 (GAP-43) (Leaver et al., 2006a). Compared to control AAV-GFP injections, only AAV-CNTF-GFP and AAV-BDNF-GFP increased RGC viability 7 weeks after ON crush. However, substantial regrowth of RGC axons distal to the crush site was only seen in rats that received AAV-CNTF-GFP injections. Similar data have been obtained after ON crush in mice, with some regenerating axons reaching the optic chiasm (Leaver et al., 2006b). In rats with PN grafts, intravitreal delivery of AAV-CNTF-GFP resulted in the survival of about 25,000 RGCs 7 weeks after the injury and about half of these neurons regrew an axon at least 1 cm into the grafts (Leaver et al., 2006a). Survival and regeneration of axons were not restricted to the transduced (GFP positive) RGC population, indicating that the secreted CNTF provided paracrine trophic support for nearby non-modified neurons.

We also found that AAV-BDNF-GFP failed to enhance the regeneration of RGC axons, even into PN grafts (Hellström and Harvey, 2011). As discussed elsewhere (Harvey et al., 2012), BDNF and the closely related neurotrophin-4/5, whether delivered by direct injection of recombinant protein or by vector-mediated methods, enhance RGC viability to a significant extent after ON injury, but neither appears to be effective in inducing long-distance regrowth of RGC axons in the adult. There is instead increased sprouting within the eye, proximal to the optic nerve injury site (Mansour-Robaey et al., 1994; Sawai et al., 1996; Cui et al., 2003; Pernet and Di Polo, 2006; Hellström and Harvey, 2011). Remarkably, in the presence of AAV-BDNF-GFP, additional intraocular injection of recombinant CNTF with a cell-permeant analogue of cAMP, 8-(4-chlorophenylthio)-adenosine-3′,5′-cyclic monophosphate (CPT-cAMP), a cocktail which typically promotes long-distance RGC axonal regeneration, failed to have any impact on the regrowth of RGC axons into a PN graft (Hellström and Harvey, 2011b). It is as if the survival promoting effects of BDNF shut down any capacity of RGCs to respond to other factors that normally re-activate long-distance axonal elongation.

In almost all AAV based gene therapy studies in the CNS, the AAV vectors are delivered 1–2 weeks prior to an injury to allow time for onset of transgene expression in infected neurons (Leaver et al., 2006a). From a translational perspective, neurotrauma in humans is almost always unexpected, unless it is a consequence of necessary neurosurgery, thus the experimental approach used in animal studies is not a useful clinical paradigm. However, by combining AAV-CNTF-GFP delivery with temporary pharmacotherapy (two intravitreal injections of rCNTF plus cAMP analogue 3 and 10 days after surgery) we have been able to develop a highly effective strategy for promoting RGC survival and the regeneration of their axons when the AAV is delivered at the time of the injury (Hellström et al., 2011b). Unlike AAV mediated delivery of CNTF, intravitreal CNTF injections upregulate suppressor of cytokine signalling (SOCS) molecules for a protracted period which lessens the impact of subsequent CNTF delivery (Park et al., 2009). AAV mediated expression of SOCS3 in RGCs prevents regeneration of their axons, even into PN grafts (Hellström et al., 2011a), consistent with other studies showing that AAV-cre deletion of SOCS3 in the eye enhances RGC regeneration (Smith et al., 2009). Interestingly, the upregulation of SOCS genes is also significantly reduced by co-injection with the cAMP analogue CPTcAMP (Park et al., 2009; Hellström et al., 2011a). Thus with this combined gene therapy and pharmacotherapeutic protocol, a mean of about 39,000 adult RGCs remained viable 4 weeks after grafting an autologous PN segment to the cut optic nerve, and about one third of these neurons regrew an axon to the distal end of the grafts.

The PN graft experiments described in the preceding paragraphs were all blind-ended, that is the distal end of the graft was placed over the dorsal aspect of the skull and not inserted back into target sites in the brain. Earlier studies that used long bridging grafts to reconnect the retina to the brain reported scattered reinnervation of target sites by regenerating RGC axons, with some limited recovery of function (Thanos, 1992; Sauvé et al., 1995; Vidal-Sanz et al., 2002). In ongoing experiments, we have repeated the retina-to-superior colliculus (SC) grafts, but with the additional use of gene therapy and pharmacotherapy, to boost RGC viability and significantly increase the number of axons that reach the distal end of the PN grafts and then grow out into the previously deafferented SC (You, Hellström, Rodger and Harvey, in preparation). Strikingly dense reinnervation has been obtained close to the PN-graft-SC interface, with the restoration of at least some simple visual behaviour when assessed through grafted eyes.

Most studies aimed at promoting neural repair and plasticity after neurotrauma appropriately focus on axonal regeneration and re-innervation, but factors that impact on axons may also affect other neuronal processes such as dendrites. For example, intraocular injection of rCNTF combined with CPT-cAMP, or injection of a pharmacological inhibitor of Rho-GTPase (BA-210) not only elicits greater axonal regrowth, but also alters the dendritic arbor patterns of regenerating RGCs (Drummond et al., 2014). Similarly, quantitative analysis of the morphology of regenerating adult rat RGCs following long term (5–8 months) AAV-mediated overexpression of BDNF or CNTF revealed significant differences in their dendritic architecture (Rodger et al., 2012). Sustained transgene expression affected not only the transduced neurons but also nearby non-transduced RGCs. We now also have data revealing long-term changes in endogenous gene expression in retinas many months after AAV-CNTF-GFP or AAV-BDNF-GFP delivery (in preparation). The stimulation of long-distance regeneration also presumably requires 'stop signals' if the actively stimulated regrowing axons are to recognize and then reinnervate appropriate target neurons in a neuropil. Together these various observations point to two elements of the therapeutic process that require refinement: i) promoters are needed that permit the regulation of transgenes, to switch them on or off when appropriate, or promoters need to be designed that are amenable to more subtle regulation, and ii) there is a need to identify and use factors that have selective effects on axonal versus dendritic compartments, and that can differentially regulate axonal elongation versus terminal arborisation and synaptogenesis.

**Alan R. Harvey**
*Corresponding author: Alan R. Harvey, Ph.D., Winthrop Professor, School of Anatomy, Physiology and Human Biology, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia, alan.harvey@uwa.edu.au.*
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