

● INVITED REVIEW

# The intricacies of neurotrophic factor therapy for retinal ganglion cell rescue in glaucoma: a case for gene therapy

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## Abstract

Regeneration of damaged retinal ganglion cells (RGC) and their axons is an important aspect of reversing vision loss in glaucoma patients. While current therapies can effectively lower intraocular pressure, they do not provide extrinsic support to RGCs to actively aid in their protection and regeneration. The unmet need could be addressed by neurotrophic factor gene therapy, where plasmid DNA, encoding neurotrophic factors, is delivered to retinal cells to maintain sufficient levels of neurotrophins in the retina. In this review, we aim to describe the intricacies in the design of the therapy including: the choice of neurotrophic factor, the site and route of administration and target cell populations for gene delivery. Furthermore, we also discuss the challenges currently being faced in RGC-related therapy development with special considerations to the existence of multiple RGC subtypes and the lack of efficient and representative *in vitro* models for rapid and reliable screening in the drug development process.

**Key Words:** glaucoma; neurotrophic factors; neural regeneration; retina; retinal ganglion cells; gene therapy; superior colliculus

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## Glaucoma: Retinal Ganglion Cells Require Neurotrophic Factors

Glaucoma, a neurodegenerative disease, is caused by the death of retinal ganglion cells (RGCs), whose axons bundle and form the optic nerve. It is the second most common cause of blindness in the world with over 70–80 million people affected by this disease. The underlying pathological mechanisms associated with glaucoma progression are still under investigation, but it is well established that the main damage to RGCs is typically the result of mechanical injury resulting from increased intraocular pressure (IOP) caused by the disruption of the trabecular meshwork. IOP reduction using various pharmacological and surgical regimens is currently the primary method of disease management. These interventions provide partial protection from the irreversible IOP-induced damage but do not actively impede the underlying neurodegenerative processes nor do they provide means to enhance ganglion cell protection and regenerative capacity. Hence, treatments that can provide effective long-term neuronal support could broaden the scope of glaucoma disease management by either preventing or repairing the damage that has already been inflicted on RGCs and the optic nerve.

It has been suggested that in glaucoma the initial damage to axonal transport in the optic nerve is caused by neurotrophic factor (NF) deficiency that is the result of a bidirectional axonal transport obstruction (**Figure 1**). Problems with anterograde flow at the optic nerve head in the retina prevent NFs synthesized by RGCs to reach their axons and disturbed retrograde transport of NFs produced in the superior colliculus (SC) in the brain to reach the RGCs (Weber et al., 2008). Evidence suggests that injections of NFs such

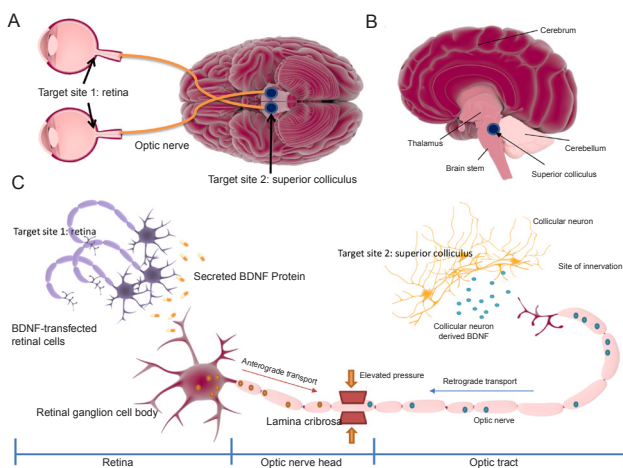
as brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), glial cell line-derived neurotrophic factor (GDNF), neurotrophin-4, and sciatic nerve (ScN)-derived factors, increase the survival of neurons in rodent models. Among the NFs, BDNF appears to provide the highest level of protection by supporting both protective and regenerative functions (see review in Alqawlaq et al., 2012). BDNF has both a direct and indirect effect on RGCs by correcting problems with the bidirectional transport of NFs and by stimulating other retinal cells' protective functions, respectively.

In the retina astrocytes, microglia and Müller cells can play a role in RGC survival through their secretion of NFs. BDNF has been identified as an attractive candidate for gene therapy applications in neuropathic diseases including glaucoma (Martin et al., 2003; Johnson et al., 2009; Ren et al., 2012). Since BDNF is supplied to RGCs by both local expression within the retina and by retrograde transport from the superior colliculus, as a logical strategy, NF therapy aims to supply and restore the local NF deficiency in the retina in order to provide RGCs with neuroprotection.

## Neurotrophic Factor Gene Therapy

### Neurotrophic factor gene delivery to the retina

In the design of delivery systems for NFs to the retina, intravitreal injectable or topical formulations could be used, however, the proteins are short-lived and sensitive to degradation en route to the retina. A better approach would be gene therapy to locally express NFs in the retina for prolonged periods of time after a single dose. Non-viral nanoparticle systems are preferred for this purpose. While nanoparticles are not equipped with intrinsic transduction machinery, resulting in



**Figure 1 Neurotrophic factor gene therapy for the protection of retinal ganglion cells (RGCs) in the retina.**

(A) Illustration of the two anatomical sites for gene therapy administration: retina and superior colliculus. (B) Superior colliculus, the site at which retinal ganglion cell axons innervate in the brain. (C) Schematic drawing describing the rationale of neurotrophic factor gene therapy and the role of retinal cells and superior collicular cells play in the cellular level. BDNF: Brain-derived neurotrophic factor.

a comparatively more challenging task to efficiently deliver plasmid DNA into the target cells compared to viral systems, viral vectors carry greater risks of immune responses as well as distribute to wide areas of the brain after a single intravitreal administration (Provost et al., 2005). Conceptually, by delivering BDNF-encoding plasmid DNA using nanoparticles to targeted cell candidates within the retina, the nanoparticle-transfected cells would be genetically modified to produce BDNF or other NFs. The BDNF-transfected cell populations could provide controlled supplies of BDNF proteins locally within the retina. An important side benefit of using nanoparticles for retinal delivery is the potential replacement of repeated intravitreal protein injections which carry risks of retinal detachment and low patient acceptance. As the main challenge lies in the sustained maintenance of BDNF level in the retina, other options such as genetically engineered retinal cells to lastingly express neurotrophic factors in the form of an implant may also provide neuroprotective support within the retina.

#### Is there a need to target the retina from two directions?

As a result of the NF supply and transport problems, under elevated IOP conditions neurotrophic support in the retina becomes insufficient. Hence, logically the retina should be the primary site for NF supplementation. However, it may be beneficial to consider the optic nerve innervation site as a secondary therapeutic target site as it may provide another means of enhancing RGC survival (Figure 1). A recent study in a cat model with mild optic nerve crush demonstrated that when BDNF protein was administered in both the eyes and the brain, RGC survival was enhanced compared to RGCs that were treated only in the eyes (Weber et al., 2010). Delivering neurotrophic factors to the site of axon innervation have two implications towards axonal repair. In a situation where tropomyosin receptor kinase B (TrkB) receptors

in the retina are downregulated and axoplasmic transport is not compromised, TrkB receptors that are present in the axons at the site of innervation can internalize exogenous BDNF and retrogradely transport it to the retina. In addition to maintaining RGC survival, BDNF may also aid in the maintenance of axon innervation to the SC, which could enhance axonal repair. While an attractive concept, delivering NFs to the superior colliculus will be even more challenging than delivery to the retina. Recent studies achieved gene expression in the retina after local administration of viral and non-viral gene delivery systems in the eye by injectable and topical methods (Di Polo et al., 1998; Alqawlaq et al., 2014). However, controlled delivery to the central nervous system (CNS) is hindered by the blood-brain barrier and several other unknown factors related to the pathways from the site of administration to the target site in the brain. Potential administration routes to reach brain targets may include both ocular and intranasal administration methods, as both have shown potential to access the brain (Han et al., 2012; Aly and Waszczak, 2015), although no studies targeting the SC has been reported so far.

#### Which ganglion cell population needs to be targeted?

Genetic heterogeneity in human populations and diseases is one of the major contributors to disease complexity and subsequent treatment outcome variability. Likewise, heterogeneity also exists within the retinal ganglion cell populations. A recent paper published by Sanes and Masland describe the existence of 30 different types of retinal ganglion cells, which are heterogeneous morphologically, physiologically, molecularly and functionally (Sanes and Masland, 2015). Moreover, different subtypes of RGCs respond to elevated intraocular pressure differently at different times (Della Santina et al., 2013). To further emphasize this, in another recent study that compared the level of survival in 11 different subtypes of retinal ganglion cells following optic nerve transection, 5 of the 11 subtypes of RGCs had a significantly higher capacity to withstand optic nerve transection (Duan et al., 2015). Having such heterogeneity within the ganglion cell population, the characterization of ganglion cells becomes ever more complex. Over the years, a number of surface- and intracellularly expressing proteins have been identified as biomarkers for RGCs, for example Thy-1, Brn3,  $\gamma$ -synuclein and  $\beta$ -III tubulin. As the diverse subtypes of RGCs are identified through a combination of histological and protein expression profiles, several key questions remain to be addressed. Importantly, from a treatment perspective, could the more resilient RGCs provide support towards the susceptible RGC populations? Perhaps a treatment targeting specific subtypes of RGCs may be more effective and provide more precise RGC protection.

#### Would synergistic approaches be necessary to regenerate the damaged optic nerve circuit?

While it is important to keep in mind that glaucoma is a multifactorial disease, NF gene therapy is a promising strategy to overcome NF deficiency-mediated pathogenesis by protecting and promoting the survival of RGCs exposed to glaucomatous stress. However, there are other aspects of glaucomatous stress that, if addressed concurrently, could

allow a more comprehensive optic nerve repair. For example, glial-mediated tissue remodeling at the lamina cribrosa (LC) under glaucomatous stress could greatly inhibit the degree of optic nerve regeneration and remyelination. LC has been indicated to be the initial site of glaucomatous damage as the thickening of the prelaminar tissue, bowing of the LC and changes to the extracellular matrices collectively result in undesirable stress on the optic nerve and axoplasmic transport obstruction. Glial cells also play an important role in the pathogenesis as the activated form of glial cells secrete various extracellular matrix proteins that contribute to the formation of scarring, which may impede the restructuring of the optic nerve (Wallace and O'Brien, 2016). In this context, gene silencing strategies to suppress fibrotic genes could be used as an add-on treatment to overcome glial scar formation. Glial cells are traditionally known for their supportive role in the CNS, however, reactive glial cells in glaucoma have been shown to play mixed roles. Despite these mixed implications, it is important not to overlook the potential benefit that glial cells could provide. A recent study found that an intraocularly injected population of genetically modified neural stem cells overexpressing CNTF preferentially differentiated into astrocytes and were neuroprotective to RGCs (Flachsbarth et al., 2014). Taken together, neurotrophic factors and glial cells both can play important roles in the regeneration of the optic nerve axons.

### Current Challenges and Future Perspectives

An ongoing issue that continues to hinder gene therapy and other therapeutic developments for RGCs is the lack of a reliable RGC model to facilitate *in vitro* screening of potential compounds or gene delivery systems in an efficient and cost-effective manner. Ideally, primary RGCs isolated from rodents or even post-mortem human retinas could serve as models, however, due to the low abundance of RGCs within the retina, the difficulty of efficient isolation, and more importantly, the short duration of isolated RGC cultures *in vitro* limits the range of testing and applications that can be performed using these primary cells. For close to a decade, the RGC-5 cells, an immortalized cell line, originally thought to be a rat RGC line, has been used as the RGC model, until recently, when it was found that the cells were misidentified and were in fact not RGCs (Krishnamoorthy et al., 2013). Evidently, a reliable RGC line is urgently needed. The need for RGCs for *in vitro* studies has inspired scientists to explore innovative ways to obtain suitable stem cells and the issue recently increased activity for the generation of suitable RGCs. Differentiation of various types of stem cells such as induced pluripotent stem cells, mesenchymal stem cells, bone-derived stem cells, and even dental pulp stem cells have been reported for successfully deriving RGCs. The use of stem cells and directing them to RGC fate opens a wide range of possibilities such as the generation of mitotic or immortalized RGCs such that proliferation can be achieved *in vitro* and that cells can be cultivated in culture for at least few passages to facilitate therapeutic screening and development.

### Conclusion

It is now well recognized that the current standard of care for lowering IOP is insufficient for glaucoma treatment. It is vital that the next generation therapies include a combination of IOP lowering treatments and local neurotrophic support within the retina with continuing efforts to develop gene therapies capable of restoring the neurotrophic balance in the retina, optic nerve and visual pathways.

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