Published in final edited form as:
Expert Rev Ophthalmol. 2011 April 1; 6(2): 165–174. doi:10.1586/eop.11.3.

Stem cell therapy for glaucoma: possibilities and practicalities

Thomas V Johnson[1,2], **Natalie D Bull**[1,3], and **Keith R Martin**[1,3,4,†]

[1]Cambridge Centre for Brain Repair, University of Cambridge ED Adrian Building Forvie Site, Robinson Way, Cambridge, CB2 OPY, UK

[2]Johns Hopkins University School of Medicine, 1830 E Monument St, Suite 2-300 Baltimore, MD 21205, USA

[3]Cambridge NIHR Biomedical Research Centre, Centre for Brain Repair, University of Cambridge ED Adrian Building Forvie Site, Robinson Way, Cambridge, CB2 OPY, UK

[4]Department of Ophthalmology, Addenbrooke's Hospital Box 41, Cambridge, CB2 0QQ, UK

Abstract

Glaucoma is a progressive, neurodegenerative, optic neuropathy in which currently available therapies cannot always prevent, and do not reverse, vision loss. Stem cell transplantation may provide a promising new avenue for treating many presently incurable degenerative conditions, including glaucoma. This article will explore the various ways in which transplantation of stem or progenitor cells may be applied for the treatment of glaucoma. We will critically discuss the translational prospects of two cell transplantation-based treatment modalities: neuroprotection and retinal ganglion cell replacement. In addition, we will identify specific questions that need to be addressed and obstacles to overcome on the path to clinical translation, and offer insight into potential strategies for approaching this goal.

Keywords

cell replacement; glaucoma; neuroprotection; optic nerve; progenitor cell; retinal ganglion cell; stem cell; transplantation

Glaucoma & the need for novel therapies

Glaucoma is a major cause of blindness in the world, affecting over 60 million people and causing bilateral blindness in over 4 million [1]. This age-related neurodegenerative condition is characterized by the progressive death of retinal ganglion cells (RGCs) and degeneration of their axons, which relay visual information to the brain via the optic nerve. Clinically, RGC loss manifests as retinal nerve fiber layer thinning with optic nerve head cupping and disc excavation. As glaucomatous visual field damage is generally painless and first affects the midperiphery, the onset and progression of glaucoma are usually insidious. Therefore, while routine ophthalmic examination is often capable of detecting glaucoma at early stages, many patients remain undiagnosed until significant functional deficits have already occurred. If left untreated, glaucoma can cause complete blindness. As in other parts

†Author for correspondence: Tel.: +44 122 333 1160, Fax: +44 122 333 1174, krgm2@cam.ac.uk.

Financial & competing interests disclosure
The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.
No writing assistance was utilized in the production of this manuscript.

of the mammalian CNS, the neural retina and optic nerve are implastic and incapable of regeneration. Thus glaucomatous vision loss is presently irreversible.

Clinical research has demonstrated that the most important treatable risk factor for the development and progression of glaucoma is elevated intraocular pressure (IOP) [2,3], and ocular hypotensive therapy remains the only clinically proven glaucoma therapy [4]. However, some patients are unresponsive to the available pharmacological and surgical ocular hypotensive treatments, while others continue to progress despite reasonable IOP control [5]. Owing to the chronic nature of the disease, patients who develop glaucoma at a relatively young age are likely to suffer significant visual deficits over the course of their lifetimes. Therefore, the development of novel treatments to halt, or even reverse, glaucomatous vision loss is urgently required.

Stem & progenitor cells

Stem cells are immature, uncommitted cell types that possess the abilities to:

- Self-renew indefinitely by symmetric cell division;
- Undergo asymmetric cell division, generating another stem cell and a daughter cell capable of differentiating into multiple mature cell types.

By definition, a cell's level of lineage commitment is inversely related to its potency. Pluripotent stem cells are capable of generating all cell types in the developing and adult body (embryonic stem [ES] and induced pluripotent stem [iPS] cells are two examples), while multipotent somatic stem cells are committed to a certain developmental lineage. During differentiation, stem cells undergo lineage commitment and lose their self-renewal capacity, thereby becoming progenitor cell that are further restricted in potency. Progenitor cells undergo limited proliferation prior to terminal differentiation to yield a mature cell type. A variety of distinct stem and progenitor cells classes exist, each with particular characteristics that make them attractive for certain potential therapeutic purposes; here, we will discuss specific cell types in the contexts to which they appear best suited. For the purposes of this article, we will not differentiate between progenitor cells and stem cells, but will use the generic term stem cell.

Stem cell transplantation therapy is of clinical interest because of its potential to treat degenerative conditions that are currently incurable (although in some cases manageable), such as glaucoma. There are at least two mechanisms by which stem cell transplantation might be applied to glaucoma. The most noteworthy, prospective therapeutic power of stem cells lies in their ability to generate new cells of many types and to effect tissue regeneration. Thus, it is conceivable that stem cells may offer therapeutic hope for glaucoma via selective cell replacement of RGCs and optic nerve regeneration to restore function [6–11]. In addition, certain types of stem cells possess protective properties capable of alleviating disease progression and promoting survival of endogenous tissue. This is proposed to occur through a variety of mechanisms, some of which are cell or tissue specific. Ideally, RGC neuroprotection would serve as an adjunct with existing ocular hypotensive therapies to prevent progressive glaucomatous vision loss [11,12]. It is likely that neuroprotective approaches to glaucoma will be clinically translated well before cell-replacement strategies, owing to far more complex requirements for the latter purpose. However, if achieved, the hope is that RGC replacement could be capable of functional vision restoration, while the aim of neuroprotection is preservation of vision. In both cases, clinical protocol development must carefully consider cell type, cell modification, route of delivery and host environmental modification, based on the intended outcome of treatment. The relative benefits, prospective logistics, and obstacles for both of these approaches are topics of this article.

It is worth noting that another potential role for stem cell transplantation in glaucoma exists. Conceivably, functional improvement might be attained by reorganizing existing retinal circuitry (e.g., expansion of RGC receptive field size might compensate for a reduction in the number of surviving RGCs) or by introducing new neural network components without overtly replacing RGCs. It is tempting to speculate that stem cell transplantation could facilitate such a repair mechanism by promoting endogenous plasticity [13] or by directly contributing to the local retinal circuitry. However, little experimental evidence for such a process currently exists, and so the remainder of this article will focus on neuroprotection and RGC replacement as avenues for applying stem cell transplantation to glaucoma.

Stem cell transplantation for RGC neuroprotection

There are currently no clinically proven neuroprotective treatments for glaucoma. However, several approaches are under investigation, including delivery of neurotrophic factors (NTFs), anti-inflammatory mediators, free radical scavengers, and other prosurvival/antiapoptotic molecules. Stem cell transplantation has been widely reported to ameliorate experimental neurodegenerative disease processes in the absence of overt functional cell replacement, and neuroprotection of endogenous host tissue has been offered as one possible explanation for this effect. It is hypothesized that transplantation of certain types of stem cells activate multiple neuroprotective pathways simultaneously via secretion of various factors [12]. In this sense, transplanted stem cells could conceivably be utilized as intraocular delivery devices for diffusible bioactive factors to achieve RGC neuroprotection in glaucoma. This approach would have the added advantage of a prolonged and localized effect, potentially mediated by multiple factors acting synergistically, and derived from a single treatment. Delivery of a single long-lasting efficacious therapy would also avoid the common problem of patient noncompliance with pharmaceutical administration.

Neurotrophic factor supplementation

Neurotophic factor deprivation has been strongly implicated in the pathophysiology of RGC death in glaucoma (recently reviewed in [14,15]). Generalized optic nerve axonal transport impairment and blockade of NTF-specific transport in the optic nerve, with relative NTF deprivation to the retina, has been observed in glaucoma models of numerous species [16–18]. In accordance with these observations, delivery of NTFs in preclinical glaucoma models by injection of purified protein [19], transplantation of slow-release devices loaded with purified protein [20,21], or viral-mediated delivery [22,23], dramatically slows the loss of RGCs. However, safe and sustained delivery of NTFs has been a stumbling block to clinical translation.

Transplantation of stem cells that secrete relatively high levels of NTFs is likely to be the most applicable short-term cell-based therapy for glaucoma. Perhaps owing to their lineage, neural stem cells secrete high levels of NTFs. Accordingly, we reported that oligodendrocyte precursor cells can protect RGCs in experimental glaucoma [24]. Other types of neural stem cells have demonstrated NTF-mediated neuroprotection of photoreceptors [25–27], although their precise relevance to RGC protection remains unevaluated. Mesenchymal stem/stromal cells (MSCs) are another stem cell type reported to secrete a battery of neurogenic signaling factors, including NTFs [28]. In contrast to neural stem cells, MSCs are therapeutically attractive because they can be isolated and expanded from bone marrow aspirates of adult patients to facilitate autologous transplantation, thereby avoiding the logistical, ethical and immunological issues associated with many other stem cell sources. Preclinical investigations have demonstrated that intravitreal transplantation of MSCs into rats with ocular hypertension, induced by either episcleral vein cauterization [29] or laser photocoagulation of the trabecular meshwork [30], offers significant RGC

protection. Previous studies of other CNS compartments also suggested that MSCs delivered intravenously 'home' sites of injury to convey neuroprotection [31–33], however we observed no such ability and no neuroprotective benefit following systemic administration in glaucoma [30]. This suggests that protocols for MSC-mediated neuroprotection in glaucoma will necessitate local administration, or will require additional manipulations to target the systemically administered cells to the eye. Homing of cells to specific organs from the peripheral circulation is a complex process that involves the combined actions of cell adhesion molecules, chemokines, chemokine receptors (including those of the CC chemokine receptor and CXC chemokine receptor families) and proteolytic enzymes [33]. Detailed characterization of chemokine expression in the normal and degenerating retina, and/or optic nerve, may shed light on potential methods for applying systemic cell delivery to retinal disease. In addition, engineering of cell-surface receptor expression prior to systemic transplantation may improve homing abilities generally [33], although retinal targeting specificity represents a challenge to be addressed by future research.

While many stem cells naturally secrete NTFs, there has also been interest in genetically modifying these cell types to increase NTF production further. Viral transduction of MSCs can dramatically increase their secretion of selected NTFs, and transplantation of these NTF-enhanced MSCs has demonstrated impressive neuroprotective efficacy in preclinical models of neurodegenerative conditions, including stroke and spinal cord injury [34–36]. However, as viral transduction may be unsafe for clinical application, alternate culture manipulations aimed at altering the phenotype of cells prior to transplantation are being investigated. For example, a culture of MSCs in a defined cocktail of factors has been shown to upregulate secretion of a variety of NTFs by MSCs [37–39]. This approach has delivered enhanced neuroprotection in several neurodegenerative models, including optic nerve transection [40]. Further investigations into methods of modulating the cellular secretome prior to transplantation could be valuable for neuroprotective applications. The ability to confer protective properties to cells by prior *in vitro* modification could broaden the potential pool from which cell-based therapies might be developed. It should be noted, however, that high levels of NTF signaling are not always preferred. Sustained, elevated NTF levels may downregulate NTF-receptor expression, thereby creating a negative feedback loop that attenuates neuroprotective effects [41]. Moreover, elevated expression of low-affinity proapoptotic NTF receptors, which reportedly occurs in glaucoma [42], can trigger apoptosis in response to NTF administration [43]. Therefore, future research must determine effective therapeutic NTF dosages to preserve RGCs in glaucoma, and assess whether cell-mediated delivery of NTFs can be titrated to achieve an effective neuroprotective dose.

Other potential mechanisms of neuroprotection

The role that the immune system plays in both promoting and ameliorating neurodegeneration, especially in glaucoma [44], is becoming increasingly appreciated. For example, the proinflammatory cytokine TNF-α is upregulated in astrocytes, Müller glia and microglia in the retina [45] and optic nerve head [46,47] of the glaucomatous eye, and may contribute to RGC death [48]. It has even been hypothesized that some forms of glaucoma represent autoimmune disorders [44,49–53]. In addition, elevated IOP triggers reactive gliosis in the retina, which is hypothesized to contribute to RGC loss in glaucoma [54,55]. Furthermore, reactive astrocytes secrete inflammatory cytokines, produce nitric oxide and reactive oxygen species [55], and have a diminished capacity to maintain tissue homeostasis.

In other CNS compartments where inflammation accompanies neurodegeneration, cell-based anti-inflammatory treatments appear to attenuate disease. In particular, MSCs demonstrate robust immunomodulatory effects and are currently under clinical trial for the treatment of multiple sclerosis [56]. In addition, neural stem cells are reportedly capable of

reducing CNS inflammation, thereby promoting functional recovery in a range of neurodegenerative diseases [57]. If inflammation proves integral to glaucomatous RGC loss, then it is conceivable that the antiinflammatory properties of transplanted stem cells could confer benefit in glaucoma.

Other purported mediators of glaucomatous neurodegeneration include oxidative stress, vascular insufficiency and excitotoxicity. There is evidence that some stem cells secrete factors that could modulate these processes. For example, hematopoetic stem cells secrete factors that modulate blood vessel development and stability, and transplantation of these cells can ameliorate some forms of retinal neurodegeneration [58]. In addition, MSCs secrete antioxidants, such as superoxide dismutase, and may thus curb oxidative stress-related neurodegeneration in certain instances [59]. However, whether these targets should be pursued via a cell-based therapy for glaucoma will depend on research to determine the extent to which these pathways are involved in glaucomatous pathophysiology, and subsequently on whether stem cells are able to adequately modulate these pathways.

It is also worth noting that while it is assumed that neuroprotection by most stem cells is conveyed by secreted factors, some cell types may confer neuroprotection via mechanisms that are dependent upon cell contact [60].

Obstacles & considerations

While many stem cell transplantation studies have demonstrated histological and functional improvement in various neurodegenerative disease models, the exact mechanism(s) and pathway(s) underlying this effect remain, for the most part, elusive. It has been hypothesized that NTF secretion, anti-inflammatory modulation and many other processes play key roles, however, definitive mechanisms specific to RGC survival in glaucoma should be elucidated prior to clinical translation. This will facilitate full comprehension of a novel treatment, and may also reveal unappreciated mechanisms of RGC neuroprotection that may be amenable to manipulation via alternate intervention.

The large secretome of many cell types is a potential challenge to fully understanding cell transplantation-mediated neuroprotective mechanisms. Undoubtedly, transplanted cells release a multitude of factors with varied bioactive effects. Preclinical models suggest that the cumulative consequence of these factors is generally beneficial for RGC survival, but the identity and relative contributions of individual factors are not yet fully understood. In addition, it is likely that deleterious factors are also released from transplanted cells. Some of these factors could curtail beneficial effects, while others might trigger off-target effects that create new problems. For example, VEGF secretion might induce retinal neovascularization. Targeted identification and elimination of these detrimental factors (if possible) might further potentiate stem cell-mediated neuroprotection, but at present any risks of such additional effects are poorly understood.

The clinical translation of preclinical studies evaluating cell-mediated neuroprotection for glaucoma is presently limited by various experimental shortcomings, including the relatively short durations of published experiments, which makes it impossible to assess the long-term efficacy of grafts. It is currently unknown whether transplanted stem cells exhibit sustained neuroprotective activity over a period of several months to years. Likewise, it has not been determined whether the host tissue remains responsive to stem cell-derived neuroprotective factors over time. Expression of certain NTF receptors is altered in chronic disease and upon extended exposure to ligand [41], thus it remains unknown whether a sustained effect can be achieved by long-term NTF supply via any delivery approach. Similar limitations may also affect the other neuroprotective mechanisms mentioned. Furthermore, timing for intervention and definition of the therapeutic window during which the host tissue is

responsive to neuroprotection require elucidation. Future research should aim to delineate the natural course of glaucomatous neurodegeneration and identify critical periods during which neuroprotective therapy would be most efficacious.

Another important experimental limitation of many published studies is a lack of functional assessment of neuroprotection. Experiments have repeatedly demonstrated that intraocular transplantation of various cell types can forestall the histological loss of retinal neurons in numerous models of neurodegenerative disease, but this effect is ultimately trivial if 'rescued' neurons are not functional. Convincing demonstrations of functional visual benefits are essential for clinical translation of stem cell therapies for glaucoma. Therefore, future studies should assess the visual function of animals following stem cell transplantation using electrophysiological and behavioral techniques.

Assuming that grafted cells offer sustained neuroprotection, long-term benefit will rely on transplant survival. Autologous transplantation is possible with some stem cell types and may reduce immunological rejection, but the long-term survival potential of grafts within the eye remains largely unknown. On the other hand, transplantation of highly proliferative cell types may carry a risk of tumorigenesis [61]. Preclinical studies suggest that this risk is relatively low provided that the cell source is not pluripotent, however, the safety of this approach must be an absolute priority in clinical translation.

Given that stem cell-mediated neuroprotection is conveyed, at least in part, via secretion of diffusible bioactive factors, it is possible that RGC protection could be attained by intravitreal transplantation of cells enclosed within an encapsulation device. Such devices permit passive diffusion of proteins and other secreted factors, while restricting the migration of both engrafted and host immune cells. An encapsulated approach would have at least three important implications for ocular cell-based therapies. The first addresses a potential drawback of intravitreal transplantation: grafted cells within the central vitreous cavity might scatter transmitted light, thereby reducing visual acuity. Implanting encapsulated cells would allow a surgeon to precisely position the graft peripherally, away from the visual axis. Second, encapsulated cells could be removed relatively easily in the case of a potential adverse event. Finally, encapsulation of cells would protect them from potential immune attack, thereby prolonging treatment efficacy following implantation. Interestingly, a device such as this, loaded with an immortalized human retinal pigment epithelial cell line engineered to deliver ciliary NTF, has been engineered [62] and is currently under Phase II/III clinical trials for retinitis pigmentosa (ClinicalTrials.gov identifiers NCT00447980 and NCT00447993 [101]) and macular degeneration (ClinicalTrials.gov identifier NCT00447954 [101]). If successful, similar approaches using other cell types should be considered for glaucoma.

Stem cell transplantation for RCG cell replacement

Prior to the seminal work of MacLaren *et al.* in 2006 [63], functional retinal neuronal replacement seemed unlikely. It is now accepted that ES and iPS cells, properly coaxed towards a photoreceptor fate, can be transplanted into the eye to generate mature rods integrated within the retina [64,65]. Of course, the steps required for stem cell transplantation-based RGC replacement are more numerous and daunting compared with those for photoreceptors. Grafted cells must differentiate into a mature RGC phenotype responsive to afferent input and capable of generating appropriate electrophysiological output. To do so, they would need to migrate into the correct spatial localization within the retinal tissue, develop mature synapses with existing retinal circuitry, generate and extend a very long axon to targets in the brain, and create efferent synapses that recapitulate the retinotopic map and preserve higher order visual processing. To date, none of these steps

have been adequately addressed and so it is easy to view the prospects of RGC replacement as science fiction. However, recent progress in three key steps on the road to RGC replacement–RGC differentiation, integration and axon regeneration provide encouragement that one day RGC replacement may be possible. We speculate that incremental advances in these areas could eventually lead to protocols capable of achieving repair of the RGC pathway, and therefore continued research in this area is critical.

RGC differentiation

A key requirement for RGC replacement therapy is the development of a reliable protocol for directing the differentiation of stem cells into RGC precursors or mature RGCs for transplantation. Analogous protocols have already been developed for the differentiation of functional retinal pigment epithelial cells from ES [66,67] and iPS cells [68,69], as well as for generating photoreceptors [64,65,70]. While convincing *in vitro* differentiation of a mature RGC from a suitable precursor cell type has yet to be demonstrated, advances in driving stem cells, including iPS cells, towards an RGC-like fate are continually being made [71–73].

At present, it is unclear whether it may be more advantageous to transplant neural or retinal stem cells, RGC precursors (committed to an RGC fate but not yet fully differentiated) or mature RGCs for RGC replacement. Experience with photoreceptor replacement suggests that an immature but committed cell type might be more inclined to integrate with existing retinal circuitry [63]. Transplantation of highly undifferentiated stem cells into the intact or lesioned mammalian retina has not yet proved particularly successful for RGC replacement. This may be due, in part, to the active suppression of RGC differentiation signaling cascades in the mature retina, or an absence of necessary receptor expression on very immature cell types. By contrast, directing differentiation of a precursor cell toward a particular lineage prior to transplantation may promote generation of the desired cellular identity and integration [63]. Transplantation of neural lineage-restricted cells has resulted in very limited expression of RGC-specific markers *in vivo* [74,75]. Thus, the ideal developmental stage required for successful integration of cellular transplants remains uncertain, and it is likely that a compromise between plasticity and maturity will be required.

Research to date also suggests that cells capable of successfully migrating into different retinal layers tend to express morphological characteristics similar to local cell types, at least when transplanted into young hosts [76]. This hints that differentiation may be modulated by local factors within the retinal microenvironment. A key to directed *in vivo* differentiation may be cell-specific depletion, and subsequent induction of a microenvironment conducive to the generation of that particular cell class. For example, *in vivo* RGC loss due to transient ischemia [77,78], or central target ablation [74], has reportedly triggered integration and limited differentiation by neural progenitor cells after their transplantation into the injured eye. Whether the glaucomatous retina can provide the necessary cues to guide the migration, differentiation and integration of transplanted cells remains to be established.

RGC integration & afferent synaptogenesis

Once suitable precursors can be reliably generated for transplantation, techniques must be optimized for maximal engraftment and integration into the host retina. *In vivo* studies have demonstrated that amalgamation of transplanted stem cells into the retina dramatically decreases with developmental age, although cells can often survive in the posterior segment of the adult eyes for weeks [76,79]. This lack of integration may be partially overcome by injuring the adult retina [75]. Nonetheless, even in cases of severe injury, suboptimal levels of retinal graft integration have been observed thus far. In addition to presenting a challenge for RGC and photoreceptor replacement in the retina, an apparent resistance of mature host

tissue to the integration of transplanted cells has also been a major impediment to neuronal replacement in the brain and spinal cord.

Research has begun to identify specific barriers to the retinal integration of grafted cells. Published data suggest that reactive gliosis represents a major impediment to stem cell migration into the neural retina [80]. Furthermore, suppression of gliotic processes can improve morphologic integration of grafted cells. The identification of particular molecules and pathways involved in establishment of this barrier should be a priority of future research. Local inflammatory cells, including activated macrophages and microglia, also impair transplanted stem cell migration into the retina, and immune suppression appears to improve graft integration, especially for xenografts [81,82]. In addition, extracellular matrix components can hamper cell migration in the CNS, as well as in the retina, and degradation of various inhibitory molecules in the microenvironment could improve graft integration [81,83]. Future research should aim to develop reliable, combinatorial methods of improving retinal graft integration following transplantation, while minimizing extraneous effects on the host retinal tissue.

Besides promoting migration of transplanted cells into the retinal ganglion cell layer, methods must be developed to encourage synaptogenesis with upstream neurons in the inner plexiform layer. While some evidence suggests that stem cell-derived photoreceptors generate efferent synapses without exogenous manipulation following transplantation [63], it is unclear whether these synapses are functional, how efficiently and exhaustively the process occurs, and whether a similar phenomenon would occur for stem cell-derived RGCs. However, it has been reported that transplanted adult hippocampal progenitor cells can localize to the retinal ganglion cell layer of the degenerating retina in Royal College of Surgeons rats, where they extended projections into the inner plexiform layer and optic nerve head [84]. Following neurite extension, synaptogenesis between numerous types of CNS neurons, including RGCs, appears to be modulated by local glial activity. Release of thrombospondins has been suggested to play a key role in this process [85] and might be further explored as a method of promoting synapse formation in the retina following transplantation.

RGC axon elongation & efferent synaptogenesis

It is perhaps the final step in RGC replacement that appears to be the greatest challenge: grafted cells must extend axons from the retina through the optic nerve to brain targets, in order to create synapses that preserve the retinotopic map and higher order visual processing. This may be especially difficult given that neurite outgrowth is inhibited within the adult retina and throughout the adult mammalian CNS. However, research into regeneration of endogenous RGC axons after injury is developing methods to overcome this inhibition. Most notably, it has been observed that some transected adult RGCs extend regenerated axons through peripheral nerve grafts [86] and that some component(s) of intraocular inflammation promote regeneration of endogenous RGC axons [87]. Furthermore, multiple laboratories have shown that combinatorial approaches that promote intrinsic RGC regeneration and suppress extrinsic inhibitory factors in the optic nerve can dramatically improve RGC axon outgrowth from RGCs after injury [88–96]. It is plausible that similar approaches could be applied to promote axon growth from stem cell-derived replacement RGCs following transplantation. While it is as yet unclear how difficult it will be for transplanted RGCs to establish functionally meaningful connections with brain targets, it is possible that some level of plasticity (occurring either as a result of the transplantation [97] or therapeutically induced) may facilitate rewiring of a regenerated visual pathway. Furthermore, it is likely that even some rudimentary reconnection would be beneficial for patients with advanced blindness and could improve quality of life.

Clearly, a tremendous amount of work remains before RGC replacement will be even theoretically plausible for patients with glaucoma, but incremental advances in stem cell biology and CNS regeneration suggest that one day such a treatment option may exist.

Obstacles & considerations

Neurodegeneration in glaucoma is not limited to the retina and optic nerve. Indeed, the death of RGCs induces downstream degenerative changes to the rest of the visual pathway. Studies in primates [98] and human patients [99] have demonstrated degeneration in the lateral geniculate nucleus as a result of glaucoma. The effects on upstream neurons (photoreceptors and bipolar cells) are less clear. Degenerative changes in the outer retina have been observed in some postmortem human glaucoma eyes [100], as well as in monkeys and rodents with experimental glaucoma [100,101], while other studies report limited photoreceptor loss associated with glaucoma [102,103]. For RGC replacement to be efficacious, the rest of the visual pathway must remain functional; upstream or downstream deficits in the glaucomatous visual pathway could limit overall functional recovery. Disease duration may also impact RGC-replacement strategies, as chronic neurodegeneration and gliotic scarring of the visual pathway are likely to reduce the capacity for therapeutic regeneration over time. Future research should address whether late-stage changes occur in the retina and/or optic nerve following overt RGC death, which may impose a therapeutic window of opportunity for RGC replacement.

Another important consideration for transplantation approaches is graft location. Some studies have suggested that the subretinal environment favors the selective differentiation of grafted cells into a photoreceptor phenotype [104], and subretinal transplants enjoy a more immune-privileged milieu than those in the vitreous. Moreover, subretinal placement ensures that the engrafted cells are held in close proximity to the retina. Alternatively, intravitreal introduction theoretically provides the transplanted cells with direct access to the inner retina. Intravitreal transplantation would also be less invasive than subretinal transplants, which induce retinal detachment. Therefore, intravitreal transplantation may prove to be more appropriate for glaucoma-directed therapy, as opposed to subretinal placement for outer retinal therapy. Both graft locations are associated with potential barriers to the integration of transplanted cells, and further research is needed to identify which transplantation technique is superior in the context of glaucoma therapy.

It is important to recognize that even if complete functional RGC replacement in glaucoma were accomplished, adjunctive ocular hypotensive and neuroprotective therapy would be required, lest the newly generated RGCs succumb to the same fate as the patient’s original cells.

Expert commentary

Stem cell transplantation represents a potential new modality for the treatment of glaucoma, with at least two potential therapeutic goals: RGC neuroprotection and RGC replacement. Recent publications have demonstrated that numerous distinct classes of stem cells, including retinal stem cells, neural stem cells and MSCs, confer protection to RGCs when transplanted intravitreally in animal models of glaucoma. While direct causal proof is currently lacking, it is generally felt that the most likely mechanisms underlying cell-mediated neuroprotection involve secretion of trophic factors and/or modulation of host inflammatory processes. Current data are limited to relatively short treatment periods, while evidence for histological protection of RGCs and the optic nerve far exceeds evidence for functional benefits. Thus, while the potential for neuroprotective stem cell transplantation in glaucoma appears promising, further research is needed before clinical translation can be seriously considered.

Retinal ganglion cell replacement represents a loftier goal for stem cell transplantation in glaucoma, with a potentially higher therapeutic payoff – that is, restoration of visual function after it has been lost. However, to date, experimental progress towards functional replacement of RGCs using exogenous transplanted cells is lacking. While functional RGC replacement, even in animal models of glaucoma, remains a major challenge, experimental progress in seemingly discrete fields of neuroscience may one day converge into a method for regenerating functional RGCs within the mature visual pathway.

It is important to recognize that whatever contribution stem cell transplantation may make in the future to the management of glaucoma, it must be carried out in conjunction with IOP reduction. Combination therapies are likely to be efficacious in preventing vision loss in glaucoma, and ideally multiple, synergistic avenues for glaucoma treatment will be available in the future. Moreover, even if RGC replacement is one day feasible, IOP must be effectively and continually managed to prevent glaucomatous degeneration of the replacement RGCs.

Five-year view

We feel that continued research should be undertaken to advance the possible application of stem cell transplantation for both neuroprotection and replacement of RGCs in glaucoma. However, in the short span of 5 years, it is likely that greater strides can be made to assess and improve the efficacy of neuroprotective protocols. Comparative studies should be carried out to identify optimal cell types/sources and to determine whether manipulation of cells prior to transplantation is necessary to enhance a graft's neuroprotective properties. Safety must also be thoroughly assessed. Encapsulation techniques should be a major focus of future research as this would improve the safety profile of potential clinical protocols. Long-term studies to evaluate the survival and safety of engrafted cells, in addition to efficacy based on histological and functional outcomes, should be undertaken.

While it is clear that RGC replacement will optimistically require decades before clinical translation can reasonably be considered, we are convinced that research efforts should continue. Great strides have been made over the past decade in many discrete but related subfields of neuroscience, such as stem cell biology, cell mobility and migration, axon regeneration, materials science and others, which can be applied to distinct steps in RGC replacement. We speculate that collaborative efforts in bringing together advancements in these isolated steps to focus on RGC replacement may eventually realize complex protocols to repair the glaucomatous visual pathway.

Key issues

- Neuroprotective therapies for glaucoma are aimed at halting or slowing the death of retinal ganglion cells and their axons in the optic nerve.
- Neuroprotective stem cell therapy for glaucoma has relatively few prerequisites: cells must simply survive and continually perform a protective activity (i.e., secretion of protective factors) while having minimal deleterious side effects.
- Stem cells might conceivably target multiple neuroprotective pathways in retinal ganglion cells and the retinal environment simultaneously, resulting in a more robust effect than could be achieved by single-factor administration.
- Stem cells offer the prospect of sustained effects following a single administration, and encapsulation of stem cells could enhance their safety profile without encroaching on the bioactivity of secreted factors.

- Future research should focus on evaluating and improving the long-term functional efficacy of neuroprotective cell transplantation protocols in preclinical glaucoma models, in an effort to approach clinical translation.
- Retinal ganglion cell replacement by stem cell therapy has numerous prerequisites including terminal differentiation, integration, axon elongation and synaptogenesis.
- Retinal ganglion cell replacement could theoretically restore functional vision, thereby serving to reverse end-stage glaucoma.
- While major gaps on the path to retinal ganglion cell replacement remain, incremental advancements in distinct steps may one day converge into clinically relevant protocols.

Acknowledgments

Thomas V Johnson is supported by a NIH OxCam Scholarship and the Johns Hopkins Medical Scientist Training Program (NIH Grant T32-GM007309). Natalie D Bull and Keith R Martin are supported by Fight for Sight (UK). Keith R Martin is also supported by the Cambridge NIHR Biomedical Research Centre and the Jukes Glaucoma Research Fund.

References

Papers of special note have been highlighted as:

• of interest

•• of considerable interest

1. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. Br. J. Ophthalmol. 2006; 90(3):262–267. [PubMed: 16488940]
2. Friedman DS, Wilson MR, Liebmann JM, Fechtner RD, Weinreb RN. An evidence-based assessment of risk factors for the progression of ocular hypertension and glaucoma. Am. J. Ophthalmol. 2004; 138 Suppl. 3:S19–S31. [PubMed: 15364049]
3. Leske MC, Heijl A, Hyman L, Bengtsson B, Dong L, Yang Z. Predictors of long-term progression in the Early Manifest Glaucoma Trial. Ophthalmology. 2007; 114(11):1965–1972. [PubMed: 17628686]
4. Maier PC, Funk J, Schwarzer G, Antes G, Falck-Ytter YT. Treatment of ocular hypertension and open angle glaucoma: meta-analysis of randomised controlled trialsq. BMJ. 2005; 331(7509):134. [PubMed: 15994659]
5. Walland MJ, Carassa RG, Goldberg I, et al. Failure of medical therapy despite normal intraocular pressure. Clin. Experiment Ophthalmol. 2006; 34(9):827–836. [PubMed: 17181612]
6. Quigley HA, Iglesia DS. Stem cells to replace the optic nerve. Eye. 2004; 18(11):1085–1088. [PubMed: 15534593]
7. Limb GA, Daniels JT, Cambrey AD, et al. Current prospects for adult stem cell-based therapies in ocular repair and regeneration. Curr. Eye Res. 2006; 31(5):381–390. [PubMed: 16714229]
8. Young MJ. Stem cells in the mammalian eye: a tool for retinal repair. APMIS. 2005; 113(11–12): 845–857. [PubMed: 16480454]
9. Bull ND, Martin KR. Optic nerve restoration: new perspectives. J. Glaucoma. 2007; 16(5):506–511. [PubMed: 17700293]
10. Bull ND, Martin KR. Using stem cells to mend the retina in ocular disease. Regen. Med. 2009; 4(6):855–864. [PubMed: 19903004]

11. Dahlmann-Noor A, Vijay S, Jayaram H, Limb A, Khaw PT. Current approaches and future prospects for stem cell rescue and regeneration of the retina and optic nerve. Can. J. Ophthalmol. 2010; 45(4):333–341. [PubMed: 20648090]
12. Bull ND, Johnson TV, Martin KR. Stem cells for neuroprotection in glaucoma. Prog. Brain Res. 2008; 173:511–519. [PubMed: 18929131]
13. Zhang Y, Klassen HJ, Tucker BA, Perez MT, Young MJ. CNS progenitor cells promote a permissive environment for neurite outgrowth via a matrix metalloproteinase-2-dependent mechanism. J. Neurosci. 2007; 27(17):4499–4506. [PubMed: 17460063] • Provides evidence that transplanted stem cells may promote endogenous neural regeneration by degrading inhibitory molecules in the extracellular environment.
14. Johnson TV, Bull ND, Martin KR. Neurotrophic factor delivery as a protective treatment for glaucoma. Exp. Eye Res. 2010 (Epub ahead of print).
15. Johnson EC, Guo Y, Cepurna WO, Morrison JC. Neurotrophin roles in retinal ganglion cell survival: lessons from rat glaucoma models. Exp. Eye Res. 2009; 88(4):808–815. [PubMed: 19217904]
16. Iwabe S, Moreno-Mendoza NA, Trigo-Tavera F, Crowder C, Garcia-Sanchez GA. Retrograde axonal transport obstruction of brain-derived neurotrophic factor (BDNF) and its TrkB receptor in the retina and optic nerve of American Cocker Spaniel dogs with spontaneous glaucoma. Vet. Ophthalmol. 2007; 10 Suppl. 1:12–19. [PubMed: 17973830]
17. Pease ME, McKinnon SJ, Quigley HA, Kerrigan-Baumrind LA, Zack DJ. Obstructed axonal transport of BDNF and its receptor TrkB in experimental glaucoma. Invest. Ophthalmol. Vis. Sci. 2000; 41(3):764–774. [PubMed: 10711692]
18. Martin KR, Quigley HA, Valenta D, Kielczewski J, Pease ME. Optic nerve dynein motor protein distribution changes with intraocular pressure elevation in a rat model of glaucoma. Exp. Eye Res. 2006; 83(2):255–262. [PubMed: 16546168]
19. Ko ML, Hu DN, Ritch R, Sharma SC, Chen CF. Patterns of retinal ganglion cell survival after brain-derived neurotrophic factor administration in hypertensive eyes of rats. Neurosci. Lett. 2001; 305(2):139–142. [PubMed: 11376903]
20. Jiang C, Moore MJ, Zhang X, Klassen H, Langer R, Young M. Intravitreal injections of GDNF-loaded biodegradable microspheres are neuroprotective in a rat model of glaucoma. Mol. Vis. 2007; 13:1783–1792. [PubMed: 17960131]
21. Ward MS, Khoobehi A, Lavik EB, Langer R, Young MJ. Neuroprotection of retinal ganglion cells in DBA/2J mice with GDNF-loaded biodegradable microspheres. J. Pharm. Sci. 2007; 96(3):558–568. [PubMed: 17177208]
22. Martin KR, Quigley HA, Zack DJ, et al. Gene therapy with brain-derived neurotrophic factor as a protection: retinal ganglion cells in a rat glaucoma model. Invest. Ophthalmol. Vis. Sci. 2003; 44(10):4357–4365. [PubMed: 14507880]
23. Pease ME, Zack DJ, Berlinicke C, et al. Effect of CNTF on retinal ganglion cell survival in experimental glaucoma. Invest. Ophthalmol. Vis. Sci. 2009; 50(5):2194–2200. [PubMed: 19060281]
24. Bull ND, Irvine KA, Franklin RJ, Martin KR. Transplanted oligodendrocyte precursor cells reduce neurodegeneration in a model of glaucoma. Invest. Ophthalmol. Vis. Sci. 2009; 50(9):4244–4253. [PubMed: 19357352]
25. Gamm DM, Wang S, Lu B, et al. Protection of visual functions by human neural progenitors in a rat model of retinal disease. PLoS ONE. 2007; 2(3):e338. [PubMed: 17396165]
26. Englund-Johansson U, Mohlin C, Liljekvist-Soltic I, Ekstrom P, Johansson K. Human neural progenitor cells promote photoreceptor survival in retinal explants. Exp. Eye Res. 2009; 90(2): 292–299. [PubMed: 19931247]
27. Wang S, Girman S, Lu B, et al. Long-term vision rescue by human neural progenitors in a rat model of photoreceptor degeneration. Invest. Ophthalmol. Vis. Sci. 2008; 49(7):3201–3206. [PubMed: 18579765]
28. Crigler L, Robey RC, Asawachaicharn A, Gaupp D, Phinney DG. Human mesenchymal stem cell subpopulations express a variety of neuro-regulatory molecules and promote neuronal cell survival and neuritogenesis. Exp. Neurol. 2006; 198(1):54–64. [PubMed: 16336965]

29. Yu S, Tanabe T, Dezawa M, Ishikawa H, Yoshimura N. Effects of bone marrow stromal cell injection in an experimental glaucoma model. Biochem. Biophys. Res. Commun. 2006; 344(4): 1071–1079. [PubMed: 16643846]
30. Johnson TV, Bull ND, Hunt DP, Marina N, Tomarev SI, Martin KR. Neuroprotective effects of intravitreal mesenchymal stem cell transplantation in experimental glaucoma. Invest. Ophthalmol. Vis. Sci. 2010; 51(4):2051–2059. [PubMed: 19933193] •• Demonstrates that intravitreal mesenchymal stem cell transplantation can ameliorate optic nerve degeneration in an ocular hypertensive rat model of glaucoma.
31. Kassis I, Grigoriadis N, Gowda-Kurkalli B, et al. Neuroprotection and immunomodulation with mesenchymal stem cells in chronic experimental autoimmune encephalomyelitis. Arch. Neurol. 2008; 65(6):753–761. [PubMed: 18541795]
32. Kim HJ, Lee JH, Kim SH. Therapeutic effects of human mesenchymal stem cells for traumatic brain injury in rats: secretion of neurotrophic factors and inhibition of apoptosis. J. Neurotrauma. 2009; 27(1):131–138. [PubMed: 19508155]
33. Karp JM, Leng Teo GS. Mesenchymal stem cell homing: the devil is in the details. Cell Stem Cell. 2009; 4(3):206–216. [PubMed: 19265660] • Nice review on the state of knowledge regarding trafficking and homing of mesenchymal stem cells from the systemic circulation to targeted organs.
34. Liu H, Honmou O, Harada K, et al. Neuroprotection by *PlGF* gene-modified human mesenchymal stem cells after cerebral ischaemia. Brain. 2006; 129(Pt 10):2734–2745. [PubMed: 16901914]
35. Ikeda N, Nonoguchi N, Zhao MZ, et al. Bone marrow stromal cells that enhanced fibroblast growth factor-2 secretion by herpes simplex virus vector improve neurological outcome after transient focal cerebral ischemia in rats. Stroke. 2005; 36(12):2725–2730. [PubMed: 16282547]
36. Sasaki M, Radtke C, Tan AM, et al. BDNF-hypersecreting human mesenchymal stem cells promote functional recovery, axonal sprouting, and protection of corticospinal neurons after spinal cord injury. J. Neurosci. 2009; 29(47):14932–14941. [PubMed: 19940189]
37. Sadan O, Shemesh N, Cohen Y, Melamed E, Offen D. Adult neurotrophic factor-secreting stem cells: a potential novel therapy for neurodegenerative diseases. Isr. Med. Assoc. J. 2009; 11(4): 201–204. [PubMed: 19603590]
38. Sadan O, Bahat-Stromza M, Barhum Y, et al. Protective effects of neurotrophic factors secreting cells in a 6OHDA rat model of Parkinson disease. Stem Cells Dev. 2009; 18(8):1179–1190. [PubMed: 19243240]
39. Sadan O, Shemesh N, Barzilay R, et al. Migration of neurotrophic factors-secreting mesenchymal stem cells toward a quinolinic acid lesion as viewed by magnetic resonance imaging. Stem Cells. 2008; 26(10):2542–2551. [PubMed: 18635865]
40. Levkovitch-Verbin H, Sadan O, Vander S, et al. Intravitreal injections of neurotrophic factors secreting mesenchymal stem cells are neuroprotective in rat eyes following optic nerve transection. Invest. Ophthalmol. Vis. Sci. 2010; 51(12):6394–6400. [PubMed: 20926814]
41. Chen H, Weber AJ. Brain-derived neurotrophic factor reduces TrkB protein and mRNA in the normal retina and following optic nerve crush in adult rats. Brain Res. 2004; 1011(1):99–106. [PubMed: 15140649]
42. Coassin M, Lambiase A, Sposato V, Micera A, Bonini S, Aloe L. Retinal p75 and bax overexpression is associated with retinal ganglion cells apoptosis in a rat model of glaucoma. Graefes Arch. Clin. Exp. Ophthalmol. 2008; 246(12):1743–1749. [PubMed: 18751719]
43. Shi Z, Birman E, Saragovi HU. Neurotrophic rationale in glaucoma: a TrkA agonist, but not NGF or a p75 antagonist, protects retinal ganglion cells *in vivo*. Dev. Neurobiol. 2007; 67(7):884–894. [PubMed: 17506493]
44. Wax MB, Tezel G. Immunoregulation of retinal ganglion cell fate in glaucoma. Exp. Eye Res. 2009; 88(4):825–830. [PubMed: 19233171]
45. Tezel G, Li LY, Patil RV, Wax MB. TNF-α and TNF-α receptor-1 in the retina of normal and glaucomatous eyes. Invest. Ophthalmol. Vis. Sci. 2001; 42(8):1787–1794. [PubMed: 11431443]
46. Yan X, Tezel G, Wax MB, Edward DP. Matrix metalloproteinases and tumor necrosis factor α in glaucomatous optic nerve head. Arch. Ophthalmol. 2000; 118(5):666–673. [PubMed: 10815159]

47. Yuan L, Neufeld AH. Tumor necrosis factor-α: a potentially neurodestructive cytokine produced by glia in the human glaucomatous optic nerve head. Glia. 2000; 32(1):42–50. [PubMed: 10975909]
48. Nakazawa T, Nakazawa C, Matsubara A, et al. Tumor necrosis factor-α mediates oligodendrocyte death and delayed retinal ganglion cell loss in a mouse model of glaucoma. J. Neurosci. 2006; 26(49):12633–12641. [PubMed: 17151265]
49. Tezel G, Wax MB. The immune system and glaucoma. Curr. Opin. Ophthalmol. 2004; 15(2):80–84. [PubMed: 15021215]
50. Tezel G, Edward DP, Wax MB. Serum autoantibodies to optic nerve head glycosaminoglycans in patients with glaucoma. Arch. Ophthalmol. 1999; 117(7):917–924. [PubMed: 10408457]
51. Maruyama I, Ohguro H, Ikeda Y. Retinal ganglion cells recognized by serum autoantibody against γ-enolase found in glaucoma patients. Invest. Ophthalmol. Vis. Sci. 2000; 41(7):1657–1665. [PubMed: 10845582]
52. Joachim SC, Pfeiffer N, Grus FH. Autoantibodies in patients with glaucoma: a comparison of IgG serum antibodies against retinal, optic nerve, and optic nerve head antigens. Graefes Arch. Clin. Exp. Ophthalmol. 2005; 243(8):817–823. [PubMed: 15834611]
53. Maruyama I, Maeda T, Okisaka S, Mizukawa A, Nakazawa M, Ohguro H. Autoantibody against neuron-specific enolase found in glaucoma patients causes retinal dysfunction *in vivo*. Jpn J. Ophthalmol. 2002; 46(1):1–12. [PubMed: 11853707]
54. Bringmann A, Pannicke T, Grosche J, et al. Müller cells in the healthy and diseased retina. Prog. Retin. Eye Res. 2006; 25(4):397–424. [PubMed: 16839797]
55. Neufeld AH, Liu B. Glaucomatous optic neuropathy: when glia misbehave. Neuroscientist. 2003; 9(6):485–495. [PubMed: 14678581]
56. Passweg J, Tyndall A. Autologous stem cell transplantation in autoimmune diseases. Semin. Hematol. 2007; 44(4):278–285. [PubMed: 17961728]
57. Martino G, Pluchino S. The therapeutic potential of neural stem cells. Nat. Rev. 2006; 7(5):395–406.
58. Otani A, Dorrell MI, Kinder K, et al. Rescue of retinal degeneration by intravitreally injected adult bone marrow-derived lineage-negative hematopoietic stem cells. J. Clin. Invest. 2004; 114(6):765–774. [PubMed: 15372100]
59. Kemp K, Hares K, Mallam E, Heesom KJ, Scolding N, Wilkins A. Mesenchymal stem cell-secreted superoxide dismutase promotes cerebellar neuronal survival. J. Neurochem. 2010; 114(6): 1569–1580. [PubMed: 20028455]
60. Jaderstad J, Jaderstad LM, Li J, et al. Communication via gap junctions underlies early functional and beneficial interactions between grafted neural stem cells and the host. Proc. Natl Acad. Sci. USA. 2010; 107(11):5184–5189. [PubMed: 20147621]
61. Arnhold S, Klein H, Semkova I, Addicks K, Schraermeyer U. Neurally selected embryonic stem cells induce tumor formation after long-term survival following engraftment into the subretinal space. Invest. Ophthalmol. Vis. Sci. 2004; 45(12):4251–4255. [PubMed: 15557428]
62. Sieving PA, Caruso RC, Tao W, et al. Ciliary neurotrophic factor (CNTF) for human retinal degeneration: Phase I trial of CNTF delivered by encapsulated cell intraocular implants. Proc. Natl Acad. Sci. USA. 2006; 103(10):3896–3901. [PubMed: 16505355] • Reports the results of a Phase I clinical trial investigating the efficacy of encapsulated cell technology for the treatment of retinitis pigmentosa. A similar approach is currently under Phase II/III clinical trials.
63. MacLaren RE, Pearson RA, MacNeil A, et al. Retinal repair by transplantation of photoreceptor precursors. Nature. 2006; 444(7116):203–207. [PubMed: 17093405] •• Landmark publication demonstrating that retinal progenitor cells isolated from the time of peak rod genesis and transplanted subretinally will integrate into the developing and adult retina and form mature rod photoreceptors, thereby providing proof-of-principle for retinal neuronal cell replacement.
64. Lamba DA, McUsic A, Hirata RK, Wang PR, Russell D, Reh TA. Generation, purification and transplantation of photoreceptors derived from human induced pluripotent stem cells. PLoS ONE. 2010; 5(1):e8763. [PubMed: 20098701]

65. Lamba DA, Gust J, Reh TA. Transplantation of human embryonic stem cell-derived photoreceptors restores some visual function in CRX-deficient mice. Cell Stem Cell. 2009; 4(1): 73–79. [PubMed: 19128794]
66. Vugler A, Carr AJ, Lawrence J, et al. Elucidating the phenomenon of HESC-derived RPE: anatomy of cell genesis, expansion and retinal transplantation. Exp. Neurol. 2008; 214(2):347–361. [PubMed: 18926821]
67. Carr AJ, Vugler A, Lawrence J, et al. Molecular characterization and functional analysis of phagocytosis by human embryonic stem cell-derived RPE cells using a novel human retinal assay. Mol. Vis. 2009; 15:283–295. [PubMed: 19204785]
68. Hirami Y, Osakada F, Takahashi K, et al. Generation of retinal cells from mouse and human induced pluripotent stem cells. Neurosci. Lett. 2009; 458(3):126–131. [PubMed: 19379795]
69. Carr AJ, Vugler AA, Hikita ST, et al. Protective effects of human iPS-derived retinal pigment epithelium cell transplantation in the retinal dystrophic rat. PLoS ONE. 2009; 4(12):e8152. [PubMed: 19997644]
70. Lamba DA, Karl MO, Ware CB, Reh TA. Efficient generation of retinal progenitor cells from human embryonic stem cells. Proc. Natl Acad. Sci. USA. 2006; 103(34):12769–12774. [PubMed: 16908856]
71. Parameswaran S, Balasubramanian S, Babai N, et al. Induced pluripotent stem cells (iPSCs) generate both retinal ganglion cells and photoreceptors: therapeutic implications in degenerative changes in glaucoma and age-related macular degeneration. Stem Cells. 2010; 28(4):695–703. [PubMed: 20166150]
72. Hegde GV, James J, Das AV, Zhao X, Bhattacharya S, Ahmad I. Characterization of early retinal progenitor microenvironment: presence of activities selective for the differentiation of retinal ganglion cells and maintenance of progenitors. Exp. Eye Res. 2007; 84(3):577–590. [PubMed: 17227675]
73. Jagatha B, Divya MS, Sanalkumar R, et al. *In vitro* differentiation of retinal ganglion-like cells from embryonic stem cell derived neural progenitors. Biochem. Biophys. Res. Commun. 2009; 380(2):230–235. [PubMed: 19167364]
74. Mellough CB, Cui Q, Spalding KL, et al. Fate of multipotent neural precursor cells transplanted into mouse retina selectively depleted of retinal ganglion cells. Exp. Neurol. 2004; 186(1):6–19. [PubMed: 14980806]
75. Nishida A, Takahashi M, Tanihara H, et al. Incorporation and differentiation of hippocampus-derived neural stem cells transplanted in injured adult rat retina. Invest. Ophthalmol. Vis. Sci. 2000; 41(13):4268–4274. [PubMed: 11095625]
76. Sakaguchi DS, Van Hoffelen SJ, Grozdanic SD, Kwon YH, Kardon RH, Young MJ. Neural progenitor cell transplants into the developing and mature central nervous system. Ann. NY Acad. Sci. 2005; 1049:118–134. [PubMed: 15965112]
77. Guo Y, Saloupis P, Shaw SJ, Rickman DW. Engraftment of adult neural progenitor cells transplanted to rat retina injured by transient ischemia. Invest. Ophthalmol. Vis. Sci. 2003; 44(7): 3194–3201. [PubMed: 12824271]
78. Kurimoto Y, Shibuki H, Kaneko Y, et al. Transplantation of adult rat hippocampus-derived neural stem cells into retina injured by transient ischemia. Neurosci. Lett. 2001; 306(1–2):57–60. [PubMed: 11403957]
79. Takahashi M, Palmer TD, Takahashi J, Gage FH. Widespread integration and survival of adult-derived neural progenitor cells in the developing optic retina. Mol. Cell Neurosci. 1998; 12(6): 340–348. [PubMed: 9888988]
80. Johnson TV, Bull ND, Martin KR. Identification of barriers to retinal engraftment of transplanted stem cells. Invest. Ophthalmol. Vis. Sci. 2010; 51(2):960–970. [PubMed: 19850833]
81. Singhal S, Lawrence JM, Bhatia B, et al. Chondroitin sulfate proteoglycans and microglia prevent migration and integration of grafted Müller stem cells into degenerating retina. Stem Cells. 2008; 26(4):1074–1082. [PubMed: 18218817]
82. Singhal S, Lawrence JM, Salt TE, Khaw PT, Limb GA. Triamcinolone attenuates macrophage/microglia accumulation associated with NMDA-induced RGC death and facilitates survival of Müller stem cell grafts. Exp. Eye Res. 2010; 90(2):308–315. [PubMed: 19961848]

83. Bull ND, Limb GA, Martin KR. Human Müller stem cell (MIO-M1) transplantation in a rat model of glaucoma: survival, differentiation, and integration. Invest. Ophthalmol. Vis. Sci. 2008; 49(8): 3449–3456. [PubMed: 18408183]
84. Young MJ, Ray J, Whiteley SJ, Klassen H, Gage FH. Neuronal differentiation and morphological integration of hippocampal progenitor cells transplanted to the retina of immature and mature dystrophic rats. Mol. Cell Neurosci. 2000; 16(3):197–205. [PubMed: 10995547]
85. Christopherson KS, Ullian EM, Stokes CC, et al. Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. Cell. 2005; 120(3):421–433. [PubMed: 15707899]
86. So KF, Aguayo AJ. Lengthy regrowth of cut axons from ganglion cells after peripheral nerve transplantation into the retina of adult rats. Brain Res. 1985; 328(2):349–354. [PubMed: 3986532]
87. Leon S, Yin Y, Nguyen J, Irwin N, Benowitz LI. Lens injury stimulates axon regeneration in the mature rat optic nerve. J. Neurosci. 2000; 20(12):4615–4626. [PubMed: 10844031]
88. Yin Y, Cui Q, Gilbert HY, et al. Oncomodulin links inflammation to optic nerve regeneration. Proc. Natl Acad. Sci. USA. 2009; 106(46):19587–19592. [PubMed: 19875691]
89. Lorber B, Howe ML, Benowitz LI, Irwin N. Mst3b, an Ste20-like kinase, regulates axon regeneration in mature CNS and PNS pathways. Nat. Neurosci. 2009; 12(11):1407–1414. [PubMed: 19855390]
90. Benowitz LI, Yin Y. Combinatorial treatments for promoting axon regeneration in the CNS: strategies for overcoming inhibitory signals and activating neurons' intrinsic growth state. Dev. Neurobiol. 2007; 67(9):1148–1165. [PubMed: 17514713]
91. Yin Y, Henzl MT, Lorber B, et al. Oncomodulin is a macrophage-derived signal for axon regeneration in retinal ganglion cells. Nat. Neurosci. 2006; 9(6):843–852. [PubMed: 16699509]
92. Fischer D, Petkova V, Thanos S, Benowitz LI. Switching mature retinal ganglion cells to a robust growth state *in vivo*: gene expression and synergy with RhoA inactivation. J. Neurosci. 2004; 24(40):8726–8740. [PubMed: 15470139]
93. Fischer D, He Z, Benowitz LI. Counteracting the Nogo receptor enhances optic nerve regeneration if retinal ganglion cells are in an active growth state. J. Neurosci. 2004; 24(7):1646–1651. [PubMed: 14973241]
94. Yin Y, Cui Q, Li Y, et al. Macrophage-derived factors stimulate optic nerve regeneration. J. Neurosci. 2003; 23(6):2284–2293. [PubMed: 12657687]
95. Moore DL, Blackmore MG, Hu Y, et al. KLF family members regulate intrinsic axon regeneration ability. Science. 2009; 326(5950):298–301. [PubMed: 19815778]
96. Park KK, Liu K, Hu Y, et al. Promoting axon regeneration in the adult CNS by modulation of the PTEN/mTOR pathway. Science. 2008; 322(5903):963–966. [PubMed: 18988856]
97. Southwell DG, Froemke RC, Alvarez-Buylla A, Stryker MP, Gandhi SP. Cortical plasticity induced by inhibitory neuron transplantation. Science. 2010; 327(5969):1145–1148. [PubMed: 20185728]
98. Ito Y, Shimazawa M, Chen YN, et al. Morphological changes in the visual pathway induced by experimental glaucoma in Japanese monkeys. Exp. Eye Res. 2009; 89(2):246–255. [PubMed: 19341728]
99. Gupta N, Greenberg G, de Tilly LN, Gray B, Polemidiotis M, Yucel YH. Atrophy of the lateral geniculate nucleus in human glaucoma detected by magnetic resonance imaging. Br. J. Ophthalmol. 2009; 93(1):56–60. [PubMed: 18697810]
100. Nork TM, Ver Hoeve JN, Poulsen GL, et al. Swelling and loss of photoreceptors in chronic human and experimental glaucomas. Arch. Ophthalmol. 2000; 118(2):235–245. [PubMed: 10676789]
101. Wang X, Tay SS, Ng YK. An electron microscopic study of neuronal degeneration and glial cell reaction in the retina of glaucomatous rats. Histol. Histopathol. 2002; 17(4):1043–1052. [PubMed: 12371131]
102. Kendell KR, Quigley HA, Kerrigan LA, Pease ME, Quigley EN. Primary openangle glaucoma is not associated with photoreceptor loss. Invest. Ophthalmol. Vis. Sci. 1995; 36(1):200–205. [PubMed: 7822147]

103. Tan O, Li G, Lu AT, Varma R, Huang D. Mapping of macular substructures with optical coherence tomography for glaucoma diagnosis. Ophthalmology. 2008; 115(6):949–956. [PubMed: 17981334]

104. Banin E, Obolensky A, Idelson M, et al. Retinal incorporation and differentiation of neural precursors derived from human embryonic stem cells. Stem Cells. 2006; 24(2):246–257. [PubMed: 16123388]

Website

201. ClinicalTrials.gov. http://clinicaltrials.gov