Cell based therapies for glaucoma

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Key take home messages:

- Stem cells could be useful in the treatment of glaucoma by improving aqueous outflow to lower eye pressure, by protecting retinal ganglion cells directly against glaucoma damage or by facilitating optic nerve regeneration.
- Stem cell treatments have been shown to reduce retinal ganglion cell death in animal models of glaucoma, demonstrating their potential to slow vision loss due to glaucoma.
- Future research may pave the way for successful transplantation of stem cells to the retina and enable differentiation into mature retinal ganglion cells able to restore functional connections to the brain, but formidable challenges remain to be overcome.

Glaucoma is the leading cause of irreversible blindness worldwide, and its global prevalence is predicted to exceed 100 million people by 2040.[1] The disease is often, but not always, associated with elevated intraocular pressure (IOP) and leads to progressive loss of retinal ganglion cells (RGCs) in the optic nerve, potentially causing blindness.[2] Current therapeutic approaches consist mainly of self-administered eye drops, surgical or laser treatment to enhance aqueous outflow and thus lower IOP. However, in many cases IOP reduction does not successfully prevent the degeneration of RGCs, and poor patient adherence to prescribed treatment regimes treatments can render them ineffective. Furthermore, symptomatic reduction in vision typically arises only late in the disease, after significant damage has been incurred. Thus, there is an urgent need for treatments which not only lower IOP and protect RGCs from dying, but also promote the growth and navigation of implanted or regenerated RGC axons through the optic nerve and restore functional vision.

Stem cells could potentially provide a compelling treatment strategy for glaucoma, either by protecting vulnerable tissues or by providing a source of mature cells to replace those lost due to disease. Stem cells are defined by their potency, that is, their potential to undergo differentiation into any cell type. At successive stages of normal development, cells enter increasingly narrow niches, moving from the totipotency of the fertilis ed egg to the multipotency of, for example, neural stem cells. Stem cells exhibit self-renewal, meaning populations can be maintained in culture, allowing for expansion of small cell numbers into robust therapeutic doses. A number of different stem cell approaches have already been used to treat a wide variety of diseases in research studies, several involving the eye. As an example, clinical trials for age-related macular degeneration and Stargardt's disease are already underway, with promising early results.[3]

Addressing glaucoma with stem cell approaches will be challenging. The retinal cell layers are intricately organised, and RGC axons must project long distances from the eye to visual targets in the brain. Replacing RGCs with exogenously derived stem cells will require careful control of the transplanted cells' differentiation state and integration into the complex architecture of the host tissue. Beyond that, new cells must then navigate the length of the optic nerve and synapse at proper brain targets. More immediate applications for stem cells in glaucoma are likely to come from endogenous sources, which may be isolated from patients' own tissue prior to autologous transplantation, limiting the risk of tumour formation. Stem cells have been shown to exert protective effects on neurons and may play a role in repairing the damaged TM. This review discusses the current spectrum of cell-based therapies for glaucoma, emphasising the need for endogenous neuroprotection and regeneration in the retina and optic nerve.

Cell-based Therapies

Aqueous Outflow Modulation

Aqueous humour fills the anterior chamber of the eye between the iris and the cornea, and the balance between production and outflow of this fluid through the TM determines IOP. Increases in the resistance of the aqueous outflow pathway, often due to decreased TM cellularity and reduced phagocytosis of debris in the extracellular matrix (ECM) as compared with healthy TM cells, contribute to elevated IOP and thus to the degeneration of RGCs.[4] Current glaucoma therapies target this pathway, using pharmacological or surgical intervention to decrease outflow resistance. Drug side effects and surgical complications therefore comprise primary risks of glaucoma treatment. Delivering a dose of stem cells that produce a sustained, long-term reduction of IOP would at least partially alleviate these concerns.

The TM occupies a space of relative immune privilege and endogenous stem cell populations have been discovered in the TM, which express characteristic markers of mature TM cells and perform a similar phagocytic function as that observed *in vivo*.[5] Other recent approaches have differentiated induced pluripotent stem (iPS) cells into TM-like cells for transplant and recovery of TM function.[6,7] TM dysfunction frequently occurs in eyes with glaucoma and often worsens with age, adversely affecting aqueous outflow and thus contributing to

IOP elevation. Restoring TM function using stem cell therapies could therefore potentially limit glaucomatous damage to RGCs and thus help prevent further vision loss in treated patients.

Retinal Neuroprotection

Transplanted stem cells can have a protective effect in the CNS, by mechanisms which may include regulation of inflammation and secretion of neurotrophic factors (NTFs). A potential therapy based on stem cell transplantation could arise from these characteristics: rather than implantation and integration of a graft in the retina, which requires exceedingly intricate control, a sustained-release approach using stem cells may directly reduce RGC death in glaucoma. A phase I clinical trial has already utilized encapsulated mesenchymal stem cells (MSCs) to deliver ciliary neurotrophic factor (CNTF) and slow retinal degeneration in patients with retinitis pigmentosa.[8] NTF deprivation likely contributes to cell death in glaucoma, and diffusible factors secreted by stem cells have shown protective effects on neurons in animal models.[9] Both MSCs and neural stem cells (NSCs) have demonstrated neuroprotection of RGCs.[10-12] Both have likewise undergone successful modifications to enhance their NTF production, although whether these modifications confer significant increases in protection remains controversial.[10,13] Despite these challenges, therapies involving stem cell-mediated neuroprotection via NTF secretion or immunomodulation have shown promise and are positioned to become an important priority for future research.

Replacement of RGCs

Conceptually, the most definitive cell-based approach to glaucoma would involve transplantation of differentiated stem cells to the damaged retina, followed by graft integration into the inner nuclear layer and projection of long axons through the optic nerve, terminating with synapses at visual targets in the brain. Naturally, each step of this complex path poses unique hurdles. Firstly, transplanted cells must migrate to the injured retina and integrate with surviving cells. This process may be impeded by local inflammatory cells, reactive Muller glia, or inhibitory molecules in the ECM.[14] Altering the gene expression of implanted cells to modify their response to external stimuli, removing inhibitory factors in the retina, or supplying exogenous NTFs may alleviate these inhibitory effects and improve graft integration.[15] Secondly, stem cells must differentiate into mature RGCs. Recent efforts have shown success in generating functional RGC-like cells in vitro from iPS cells.[16] Such cells have yet to demonstrate a differentiated RGC-like phenotype *in vivo*.

Even if grafted cells survive and differentiate into RGCs, they must then navigate the inhibitory environment of the optic pathway and generate synapses at visual targets that maintain the retinotopic map of the retina. Most research on axon projection derives from regeneration studies of endogenous RGCs, and the behaviour of transplanted cells may not mirror that of surviving cells. Nonetheless, several groups have shown success in using combinatorial treatments to stimulate RGC axon regeneration in animal models. These typically include both intrinsic methods such as genetic manipulation of RGCs, and extrinsic changes including modification of inhibitory ECM components, peripheral nerve grafts, or inflammatory stimulation. One such experimental approach yielded full-length regeneration of a small population of neurons in mice, with reported gains in visual function and behaviour.[17]

Future Directions

For short-term clinical impact, stem cell-based therapies for glaucoma will most likely rely on proven effects of IOP lowering and retinal neuroprotection. Optimising these treatments may slow degeneration of RGCs and ensure that a larger proportion of cells survive for longer periods. Stimulating regeneration of these surviving cells could then provide a mechanism by which visual function, even at relatively low levels, may be recovered, thus dramatically improving the quality of life for patients with severe vision loss. As these studies move forward, it may become possible to facilitate exogenous transplantation and integration of new RGCs with active connections to the brain, although many formidable challenges remain to be overcome.

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REFERENCES

1. Tham YC, Li X., Wong TY, Quigley HA, Aung T, Cheng CY. *Global Prevalence of Glaucoma and Projections of Glaucoma Burden through 2040: A Systematic Review and Meta-Analysis.* Ophthalmology 2014;1–10.
2. Weinreb RN, Aung T, Medeiros FA. *The pathophysiology and treatment of glaucoma: a review.* JAMA J. Am. Med. Assoc. 2014;311:1901–11.
3. Schwartz SD, et al. *Embryonic stem cell trials for macular degeneration: a preliminary report.* Lancet 2012;379:713–20.
4. Zhang X, Ognibene CM, Clark AF, Yorio T. *Dexamethasone inhibition of trabecular meshwork cell phagocytosis and its modulation by glucocorticoid receptor B.* Exp. Eye Res.2007; 84:275–84.
5. Du Y, Roh DS, Mann MM, Funderburgh ML, Funderburgh JL, Schuman JS. *Multipotent stem cells from trabecular meshwork become phagocytic TM cells.* Investig. Ophthalmol. Vis. Sci. 2012;53:1566–75.
6. Ding QJ, Zhu W, Cook AC, Anfinson KR, Tucker BA, Kuehn MH. *Induction of trabecular meshwork cells from induced pluripotent stem cells.* Investig. Ophthalmol. Vis. Sci. 2014;55:7065–72.
7. Abu-Hassan DW, Li X, Ryan EI, Acott TS, Kelley MJ. *Induced pluripotent stem cells restore function in a human cell loss model of open-angle glaucoma.* Stem Cells 2014:1–14.
8. Sieving PA, Caruso RC, Tao W, Coleman HR, Thompson DJS, Fullmer KR, Bush RA. *Ciliary neurotrophic factor (CNTF) for human retinal degeneration: phase I trial of CNTF delivered by encapsulated cell intraocular implants.* Proc. Natl. Acad. Sci. U. S. A. 2006:103,:3896–901.
9. Martin KRG, 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;83:255–62.
10. Flachsbarth K, Kruszewski K, Jung G, Jankowiak W, Riecken K, Wagenfeld L, Richard, G, Fehse B, Bartsch U. *Neural Stem Cell-Based Intraocular Administration of Ciliary Neurotrophic Factor Attenuates the Loss of Axotomized Ganglion Cells in Adult Mice.* Investig. Ophthalmol. Vis. Sci. 2014;55:7029–39.
11. Emre E, Yüksel EN, Duruksu G, Pirhan D. *Neuroprotective effects of intravitreally transplanted adipose tissue and bone marrow-derived mesenchymal stem cells in an experimental ocular hypertension model.* Cytotherapy 2015;1–17.
12. Johnson TV et al. *Identification of retinal ganglion cell neuroprotection conferred by platelet-derived growth factor through analysis of the mesenchymal stem cell secretome.* Brain 2014;137:503–19.
13. Harper MM, Grozdanic SD, Blits B, Kuehn MH, Zamzow D, Buss JE, Kardon RH, Sakaguchi DS. *Transplantation of BDNF-secreting mesenchymal stem cells provides neuroprotection in chronically hypertensive rat eyes.* Investig. Ophthalmol. Vis. Sci. 2011:52;4506–15.
14. Johnson TV, Bull ND, Martin KR. *Identification of barriers to retinal engraftment of transplanted stem cells.* Investig. Ophthalmol. Vis. Sci. 2010;51:960–70.
15. Jiao Y, Palmgren B, Novozhilova E, Johansson UE, Spieles-engemann AL, Kale A, Stupp SI, Olivius P. *BDNF Increases Survival and Neuronal Differentiation of Human Neural Precursor Cells Cotransplanted with a Nanofiber Gel to the Auditory Nerve in a Rat Model of Neuronal Damage.* Biomed Res. Int. (2014).
16. Tanaka T, Yokoi T, Tamalu F, Watanabe SI, Nishina S, Azuma N. *Generation of retinal ganglion cells with functional axons from human induced pluripotent stem cells.* Sci. Rep. 2015;5:1–11.
17. De Lima S et al. *Full-length axon regeneration in the adult mouse optic nerve and partial recovery of simple visual behaviors.* Proc. Natl. Acad. Sci. 2012:109:13465.