Special Issue

The Potential of Human Stem Cells for the Study and Treatment of Glaucoma

Xitiz Chamling,[1] Valentin M. Sluch,[1,2] and Donald J. Zack[1–4]

[1]Department of Ophthalmology, Wilmer Eye Institute, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States
[2]Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States
[3]The Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States
[4]Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States

Correspondence: Donald J. Zack, Johns Hopkins University School of Medicine, 3029 Smith Building, 400 N. Broadway, Baltimore, MD 21231, USA;
dzack@jhmi.edu.

Submitted: November 5, 2015
Accepted: January 5, 2016

Citation: Chamling X, Sluch VM, Zack DJ. The potential of human stem cells for the study and treatment of glaucoma. *Invest Ophthalmol Vis Sci*. 2016;57:ORSFi1–ORSFi6. DOI:10.1167/iovs.15-18590

Purpose. Currently, the only available and approved treatments for glaucoma are various pharmacologic, laser-based, and surgical procedures that lower IOP. Although these treatments can be effective, they are not always sufficient, and they cannot restore vision that has already been lost. The goal of this review is to briefly assess current developments in the application of stem cell biology to the study and treatment of glaucoma and other forms of optic neuropathy.

Methods. A combined literature review and summary of the glaucoma-related discussion at the 2015 "Sight Restoration Through Stem Cell Therapy" meeting that was sponsored by the Ocular Research Symposia Foundation (ORSF).

Results. Ongoing advancements in basic and eye-related developmental biology have enabled researchers to direct murine and human stem cells along specific developmental paths and to differentiate them into a variety of ocular cell types of interest. The most advanced of these efforts involve the differentiation of stem cells into retinal pigment epithelial cells, work that has led to the initiation of several human trials. More related to the glaucoma field, there have been recent advances in developing protocols for differentiation of stem cells into trabecular meshwork and retinal ganglion cells. Additionally, efforts are being made to generate stem cell–derived cells that can be used to secrete neuroprotective factors.

Conclusions. Advancing stem cell technology provides opportunities to improve our understanding of glaucoma-related biology and develop models for drug development, and offers the possibility of cell-based therapies to restore sight to patients who have already lost vision.

Keywords: stem cells, glaucoma, ganglion cells, RGC differentiation, neuroprotection

Glaucoma is a debilitating disease that leads to slow, painless loss of vision. It is marked by cupping and structural damage at the optic nerve head, and it is characterized by pathological features such as thinning of the neuroretinal rim and the retinal nerve fiber layer.[1,2] Vision loss in glaucoma generally starts in the periphery, but as the disease advances it moves centrally, and can eventually result in total blindness. In fact, it is the second leading cause of blindness worldwide.[3] The best currently understood and treatable risk factor for glaucoma is the eye's intraocular pressure (IOP).[4] Despite the importance of controlling IOP, the ultimate cause of glaucoma-associated vision loss is axonal damage and progressive loss of retinal ganglion cells (RGCs), the retinal neurons whose axons make up the optic nerve and transmit visual information from the eye to the brain.

Human RGCs are postmitotic neurons that do not regenerate, and as a result the vision loss sustained from their death is irreversible. A further challenge is that significant retinal damage and cell death occurs in glaucoma before the patient experiences detectable visual field loss, making early detection and treatment difficult.[5,6] In spite of these challenges, recent exciting discoveries in the ocular regenerative and related fields raise the possibility that in future years it may be possible to use cell-based approaches to restore vision in patients suffering from glaucoma and other forms of optic nerve diseases. Here, we will review some of these advances in the ocular stem cell field that will hopefully aid in our understanding of the mechanisms of damage in glaucoma, provide tools for the discovery of neuroprotective drugs and other novel therapeutic agents for the treatment of the optic nerve diseases, and potentially lead to the development of cell-based approaches for vision restoration. Although we will briefly mention some work on stem cell–derived trabecular meshwork (TM) cells and their potential as a new approach for IOP control, this review will focus mainly on the use of stem cell–derived RGCs for drug discovery and transplantation-based therapy. This review is based on discussion at a meeting entitled "Sight Restoration Through Stem Cell Therapy" held on June 13, 2015 in Santa

Monica, California, United States, that was sponsored by the Ocular Research Symposia Foundation (ORSF).

Stem Cell–Derived Trabecular Meshwork Cells: Control of Intraocular Pressure

Depending on the anatomy of the anterior chamber, glaucoma is broadly categorized as open or closed angle.[4] Insufficient drainage of aqueous humor in both cases can lead to increased IOP. Essentially all drugs currently available for glaucoma treatment lower IOP by modifying aqueous dynamics, by inhibiting aqueous production by the ciliary body and/or by increasing aqueous outflow, through the trabecular and/or uveoscleral outflow pathways.[7] Trabecular outflow involves fluid passage through the fenestrated beams of the TM, an area of tissue located in the anterior chamber in close proximity to the ciliary body.[8] The TM plays an important role in determining the resistance to aqueous humor outflow,[9] and loss of TM cells has been shown to compromise IOP homeostasis.[10] Mechanical stress, insults, and injuries can lead to death or dysfunction of TM cells.[11] For example, during progression of POAG, IOP increase has been linked to oxidative stress, to which the TM cells are very susceptible.[12,13] Damage to the TM can result in changes in cell morphology, attenuation of its ability to maintain aqueous flow and phagocytize unwanted cellular material, and can also trigger apoptosis. Through these and other mechanisms, dysfunction and death of TM cells can lead to progressive IOP increase and initiation of a "glaucomatous cascade" leading to RGC degeneration. Although some glaucoma drugs can stimulate aqueous outflow through the TM, no therapies are currently available to improve the health of or replace damaged or senescent TM cells in POAG patients. Through stem cell technology it may be possible to develop such therapies.

The presence of stem cells in the TM has been suspected for a number of years.[14–16] and attempts have been made to characterize these putative cells.[17] Recently, mesenchymal stem cells (MSCs) isolated from human TM samples were successfully propagated in vitro.[18,19] Interestingly, these TM-derived stem cells were capable of differentiating into phagocytic TM cells in vitro, as well as in vivo when transplanted into wild-type mice.[19,20] Injection of bone marrow–derived mouse MSC has also been shown to mediate regeneration of damaged TM in vivo, apparently through production of paracrine factors.[21] It would be interesting to test if human TM–derived stem cells are also capable of secreting analogous factors and mediating in vivo regeneration of TM cells in mice, and eventually in humans. If the relevant factors are more fully defined, it might also be possible to provide them either by a gene therapy approach or by a transplantation approach using cells that have been engineered to secrete the relevant factors.

Successful differentiation of TM cells from mouse induced pluripotent stem cells (iPSCs) as well as human iPSCs has also been reported. Ding et al.[22] performed a coculture of mouse iPSCs with primary human TM to achieve TM-like cells, and Abu-hassan et al.[10] used TM cell–derived extra cellular matrix (ECM) and TM cell–derived conditioned media to achieve TM differentiation from human iPSCs. In their encouraging study, Abu-Hassan et al.[10] further reported that when transplanted into an ex vivo human TM cell loss model, the iPSC derived TM-like cells resembled endogenous TM cells and successfully restored, at least partially, IOP homeostatic function. This result suggests the promising possibility of using stem cell–derived TM cells as a cell-based therapy to restore IOP regulatory function in open-angle glaucoma patients, which should aid in the preservation of remaining optic nerve function. In addition, the availability of SC-derived human TM cells could provide a powerful resource to screen and discover novel pathways and therapeutic drugs that could target the TM to reduce IOP.

Pluripotent Stem Cells as a Source of RGCs

In addition to their potential role in helping to control IOP, an even more exciting and transformative role for stem cells in glaucoma research and treatment is their possible use in the direct preservation and restoration of RGC function. As noted above, it is the damage and loss of RGC axons and cell bodies that ultimately leads to vision loss. Encouragingly, a number of signaling pathways and neuroprotective compounds that promote RGC function and survival in cell culture or animal models have been reported,[1,23–29] but none have yet successfully made it to the clinic.

In the developing mammalian eye, RGCs arise from retinal progenitor cells (RPCs), a multipotent cell type that differentiates to the six major neuronal cell types of the retina and to Müller glia.[30–32] Retinal cells are born in chronologic order, in a highly conserved but overlapping manner. Retinal ganglion cells are the first cells to arise from RPCs followed by horizontal cells, cone photoreceptors, amacrine cells, bipolar cells, rod photoreceptors, and Müller glia.[33] Coordinated expression of multiple transcription factors is involved in specifying retinal cell fate. For example, *Pax6* is one of the early transcription factors required to maintain the multipotency and competence of RPCs and to generate all retinal cell types except amacrine cells.[34] The basic helix-loop-helix (bHLH) transcription factor *Atoh7* (*Math5*) is required for RGC specification and subsequent expression of the POU-homeodomain transcription factor *Brn3b*.[35,36]

Similar to retinal development in vivo, RGCs are the first retinal cells to be born during in vitro differentiation of retinal cells from stem cells, and hence they offer a promising area for research. However, for technical and other reasons, efforts at RGC differentiation from stem cells have gained less attention and have had limited success compared to the differentiation of retinal pigment epithelium (RPE) and photoreceptor cells. In addition to the transcription factors mentioned above, retinogenesis involves a number of highly regulated signaling pathways, such as FGF,[37] insulin-like growth factor (IGF),[38] epidermal growth factor (EGF), bone morphogenetic protein (BMP),[39] sonic hedgehog (SHH), Wnt,[40] Notch, and Nodal.[32,41] Researchers have been able to use cocktails of growth factors and small molecules (e.g., FGF1, FGF2, IGF1, DKK [Dickkopf family protein and inhibitor of canonical Wnt pathway], and DAPT [inhibitor of Notch signaling pathway])[32] to manipulate these signaling pathways and direct the differentiation of various retinal cell types. Boucherie et al.[42] reported retinal differentiation using extra cellular matrix signals provided by Matrigel, which eliminates tedious embryoid body formation and suspension culture. En route to differentiating photoreceptors and other later-born retinal cells, differentiation of RGC-like cells has been reported,[43,44] but only a few protocols have focused on RGC differentiation.[45–47]

Retinal ganglion cell generation in three dimensional (3D) culture systems has also attracted some attention. In a 3D self-organization technique, intrinsic cellular programs drive self-organization of stem cells to form an optic cup, which can further differentiate into a multilayered structure containing various retinal cells.[48,49] By optimizing the 3D culture technique, Maekawa et al.[47] reported successful RGC neurite outgrowth from the 3D optic vesicles.

Investigative Ophthalmology & Visual Science

Although the above described protocols for RGC differentiation from murine[46] and human[45,47] stem cells are promising, they yield heterogeneous retinal cell populations with limited numbers of RGCs. The ability to generate homogeneous RGC populations would be desirable for a number of reasons, such as use in biochemical assays, disease modeling, and transplantation studies. With this goal in mind, we have modified the photoreceptor-directed protocol of Boucherie et al.[42] and used CRISPR-Cas9 genome editing technology to generate a human ES *BRN3B* mCherry fluorescent reporter cell line that can be used with fluorescence activated cell sorting (FACS) to generate highly purified populations of functional RGCs.[50] Additional work is ongoing to further improve efficiency and yield. High-yield generation of motor neurons[51] and efficient neural conversion of human embryonic stem cells (hESCs)[52] have been achieved using small molecules, and it is hoped that parallel approaches will yield similar improvements in the speed and extent of RGC differentiation and the ability to generate RGCs of distinct subtypes.

Stem Cell–Derived RGCs for Transplantation and Vision Restoration

The progress being made on transplantation of RPE[53–56] and photoreceptor[57–59] cells in combination with the above described advances in RGC differentiation protocols, is encouraging and suggests that in the coming years RGC transplantation could progress from a dream to an achievable reality. However, much work remains to be done, as successful transplantation of RGCs is a more formidable challenge than transplanting RPE or photoreceptor cells. Even though photoreceptor transplantation will also have the challenge of forming correct synapses to bipolar and horizontal cells, such synapses will be local in nature while for RGCs the axons will have to navigate the nerve fiber layer, optic disc, optic nerve, and chiasm, and travel long distances to reach their appropriate target tissues. And even if they arrive at the correct terminal regions (lateral geniculate nucleus [LGN] or other secondary synaptic sites), they will still need to synapse to appropriate higher order neurons to achieve meaningful spatial-temporal vision. To add further complexity, it is essential to consider that normal RGC axonal guidance and synaptogenesis occurs during development,[60] while in glaucoma, due to the natural history of the disease, transplanted RGCs will need to recapitulate the process in an adult environment, which may lack the proper guidance cues, both attractive and repulsive, that are present during development.[60–62]

While the nature and status of axonal pathfinding cues in the adult environment are not well understood,[63] there are some encouraging data, using a combination of genetic alteration and stimulation of signaling pathways, that suggest that the process of optic nerve regeneration, at least in the mouse, may be possible.[64–66] In these recent studies, RGC axons following optic nerve crush injury were able to regenerate all the way to the brain, and they apparently restored some limited visual functions, such as optomotor response and circadian photoentrainment.[64] These results suggest that RGC regeneration is, at least in part, a cell autonomous process and that regenerating RGCs, with the proper manipulation, may be able to overcome inhibitory guidance cues and find appropriate targets. As encouraging as these results are, it should be noted that the fraction of cells reaching their target was very small, and the visual phenomenon measured was very crude compared with what would be needed to restore useful human vision. Additionally, due to the likely hostile environment of a degenerating retina, it may be necessary to combine transplantation with neuroprotective[1,29] (see below) and anti-inflammatory[67] strategies to promote survival and quell the potentially negative inflammatory response.

Additional encouraging areas of research involve studies of lower vertebrates, such as frogs and fish that are able to fully and functionally regenerate their optic nerves.[68] Based on studies of such organisms, it will hopefully be possible to learn "tricks" to help mammalian RGCs regenerate. Additionally, it may be possible to engineer artificial scaffolds to guide RGC axons to their proper brain targets.[69,70] Through combinatorial approaches involving genetic engineering of pluripotent stem cells, differentiation of such cells into RGCs, and the incorporation of bioengineering guidance scaffolds, it may be possible to direct stem cell–derived RGCs to integrate into the retina, repopulate the optic nerve with new axons, and then reach and synapse with brain targets to make visual recovery possible. Although there have not yet been any reports of stem cell–derived RGC axon growth into the optic nerve, a parallel experiment has been reported describing mouse embryo–derived retinal precursor cell transplantation into the adult mouse, demonstrating a limited but clear donor RGC contribution to the optic nerve.[71]

In addition to biological intervention for retinal regeneration artificial prosthetic systems for inducing electrical stimulation of the retina have also been developed, which rely on stimulating retinal circuitry downstream of photoreceptors.[72,73] Because visual perception is a complex process that it is not yet understood well enough on an electrical scale, these artificial systems can only provide rudimentary perception of light rather than true vision, but there is hope that this technology will continue to evolve. Such prosthetic systems are more difficult to implement in a context of RGC loss, but deep brain stimulation in areas of the visual cortex and other brain regions has been proposed as a possible means of bypassing the optic nerve altogether for visual perception recovery.[74]

Although it is definitely a daunting task, RGC transplantation may prove to be more effective than prosthetic technologies because direct brain stimulation represents a risky procedure and it still remains to be seen whether a computer will be able to recapitulate retinal function and properly communicate with the brain for visual processing. Retinal ganglion cell transplantation carries its own risks to be sure, as inappropriate rewiring of the brain's visual centers could result in disturbance of brain function or pain, but RGCs themselves are relatively unlikely to lead to tumor growth due to their postmitotic nature. Furthermore, in the unlikely event of a transplanted RGC-associated tumor, the newly formed optic nerve could be surgically removed to protect the brain from harm, although of course at the cost of losing any residual vision. Regardless of which strategy becomes most tractable, patients suffering from blindness due to optic nerve disease currently do not have any options for vision restoration. As such, it remains an important goal to develop novel approaches to replace lost neurons as well as to integrate them into the working visual system.

Stem Cells as a Source for Neurotrophic Factors

Deprivation of the neurotrophic factor (NTF) required for the maintenance and survival of neurons in the optic nerve has been suggested to play a role in the progression of RGC damage in glaucoma. Elevation of IOP has been reported to obstruct the retrograde transport of NTFs such as brain-derived neurotrophic factor (BDNF)[75,76] and NT-4/5[77] to the RGC soma, and the deprivation of these NTFs can induce RGC

Investigative Ophthalmology & Visual Science

apoptosis. Supplementing additional BDNF and other NTFs such as glial cell–derived neurotrophic factor (GDNF),[25] ciliary-neurotrophic factor (CNTF),[23,26] and IGF-1[78] have been reported to promote RGC survival in vitro and in vivo. However, delivery of NTFs to the retina and RGCs is challenging because access to the neural retinal tissue requires circumventing of the blood–retinal barrier. Systemic administration would either fail to reach the neural tissues or not provide high enough doses for the NTFs to have a positive effect. Intravitreal injections of BDNF have been shown to enhance RGC survival in rats with chronic IOP elevation,[79] but long-term delivery through multiple injections is unlikely to be well tolerated by patients for a disease such as glaucoma.

Stem cells could provide a viable approach for long-term NTF delivery to RGCs. Mesenchymal stem cells derived from the bone marrow (hBMSC) of adult patients were shown to secrete several neurotrophic factors (NTFs), such as BDNF and β-NGF, and promote neuronal cell survival and neuritogenesis in vitro as well as in vivo.[24] In another study, secretion of NGF, BDNF, and VEGF by hBMSC, human dental pulp stem cells (hDPSC), and human adipose–derived stem cells (hAMSC) were tested and the role of these stem cells in neuroprotection was analyzed. In comparison with hBMSC and hAMSC, hDPSC was reported to produce higher levels of those growth factors and promote significantly more neuroprotection in vitro.[27] In an in vivo assay, Harper et al.[80] transplanted MSCs engineered to produce and secrete BDNF into a chronic ocular hypertension rat, and they reported preservation of RGCs and optic nerve function in the BDNF-MSC–treated eyes. In a recent study, Ma et al.[28] engineered human neural progenitor cells (hNP), which intrinsically target the inner retina layer,[81] to produce IGF-1 and transplanted them by intravitreal injection. The transgenic hNP cells successfully entered the inner retinal layer and provided neurotrophic support to prevent RGC loss.[28] Overall, these findings provide compelling evidence for stem cell–based local delivery of neurotrophic factors for neuroprotection.

Cell encapsulation and transplantation is another promising, alternative method for NTF delivery.[82] Tao et al.[83] encapsulated mammalian cells genetically engineered to produce CNTF and implanted them into the vitreous humor of a canine retinitis pigmentosa model. The encapsulated cells remained viable, produced low levels of CNTF, and enhanced the survival of photoreceptor cells.[83] However, although encouraging in animal models, these Neurotech CNTF producing encapsulated cells in human clinical trials for retinal degenerative disease have demonstrated equivocal efficacy, at best.[84] A human glaucoma clinical trial using these encapsulated cells is also underway, but no clinical efficacy results have been reported to date.[85] As an alternative to the encapsulated RPE cell line used in the Neurotech implant, stem cells could be engineered or differentiated to produce the desired NTFs, encapsulated, and transplanted into the vitreous of an eye, or unencapsulated NTP-producing retinal cells differentiated from stem cells could be transplanted and allowed to integrate into the inner retina.

Disease Modeling With hiPSCs and Genome Editing

Genetic study of glaucoma has identified more than 20 genetic loci associated with the disease. At least three causative genes (*Myocilin*, *Optineurin*, and *WDR36*) and several risk factor genes (*CAV1/CAV2*, *CDKN2B*, *TMCO1*, *SIX1/SIX6*, and *LRP12/ZFPM2*, *TBK1*, and *GALC*) have been reported to date.[86] However, how the mutations in these genes cause disease or increase the risk of developing glaucoma is not well understood. Biochemical studies to test the effects of these mutations on target cell types such as TM and RGCs have been difficult because fresh retinal tissues from patients are not easily obtainable, and tissues with the desired genotypes are particularly hard to obtain. The ability to obtain TM cells and RGCs from ES and iPS cells, together with the remarkable power of CRISPR/Cas9 genome editing, may greatly assist the study of the mechanisms by which genetic factors modulate the risk of developing glaucoma.

In summary, although much still remains to be learned, and important and difficult challenges remain, the increasing pace of ocular stem cell biology research is very exciting, and the field offers great promise for increasing our understanding of glaucoma pathogenesis and for developing new and improved treatment approaches.

Acknowledgments

Supported by BrightFocus Foundation, The Glaucoma Foundation, Research to Prevent Blindness, and the Guerrieri Family Foundation.

Disclosure: **X. Chamling**, None; **V.M. Sluch**, None; **D.J. Zack**, None

References

1. Chang EE, Goldberg JL. Glaucoma 2.0: neuroprotection, neuroregeneration, neuroenhancement. *Ophthalmology*. 2012;119:979–986.
2. Quigley HA. Open-angle glaucoma. *New Engl J Med*. 1993; 328:1097–1106.
3. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol*. 2006;90:262–267.
4. The Advanced Glaucoma Intervention Study (AGIS): 7. The relationship between control of intraocular pressure and visual field deterioration. The AGIS Investigators. *Am J Ophthalmol*. 2000;130:429–440.
5. Harwerth RS, Carter-Dawson L, Shen F, Smith EL III, Crawford ML. Ganglion cell losses underlying visual field defects from experimental glaucoma. *Invest Ophthalmol Vis Sci*. 1999;40: 2242–2250.
6. Quigley HA, Addicks EM, Green WR. Optic nerve damage in human glaucoma. III. Quantitative correlation of nerve fiber loss and visual field defect in glaucoma, ischemic neuropathy, papilledema, and toxic neuropathy. *Arch Ophthalmol*. 1982; 100:135–146.
7. Toris CB. Pharmacotherapies for glaucoma. *Curr Mol Med*. 2010;10:824–840.
8. Acott TS, Kelley MJ. Extracellular matrix in the trabecular meshwork. *Exp Eye Res*. 2008;86:543–561.
9. Acott TS, Kelley MJ, Keller KE, et al. Intraocular pressure homeostasis: maintaining balance in a high-pressure environment. *J Ocul Pharmacol Ther*. 2014;30:94–101.
10. 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*. 2015;33:751–761.
11. Kelley MJ, Rose AY, Keller KE, Hessle H, Samples JR, Acott TS. Stem cells in the trabecular meshwork: present and future promises. *Exp Eye Res*. 2009;88:747–751.
12. Sacca SC, Izzotti A. Oxidative stress and glaucoma: injury in the anterior segment of the eye. *Prog Brain Res*. 2008;173: 385–407.
13. Izzotti A, Sacca SC, Cartiglia C, De Flora S. Oxidative deoxyribonucleic acid damage in the eyes of glaucoma patients. *Am J Med*. 2003;114:638–646.

14. Raviola G. Schwalbe line's cells: a new cell type in the trabecular meshwork of Macaca mulatta. *Invest Ophthalmol Vis Sci*. 1982;22:45–56.
15. Melamed S. Trabecular repopulation by anterior trabecular meshwork cells after laser trabeculoplasty. *Am J Ophthalmol*. 1989;108:209–210.
16. Acott TS, Samples JR, Bradley JM, Bacon DR, Bylsma SS, Van Buskirk EM. Trabecular repopulation by anterior trabecular meshwork cells after laser trabeculoplasty. *Am J Ophthalmol*. 1989;107:1–6.
17. Gonzalez P, Epstein DL, Luna C, Liton PB. Characterization of free-floating spheres from human trabecular meshwork (HTM) cell culture in vitro. *Exp Eye Res*. 2006;82:959–967.
18. Tay CY, Sathiyanathan P, Chu SW, Stanton LW, Wong TT. Identification and characterization of mesenchymal stem cells derived from the trabecular meshwork of the human eye. *Stem Cells Dev*. 2012;21:1381–1390.
19. Du Y, Roh DS, Mann MM, Funderburgh ML, Funderburgh JL, Schuman JS. Multipotent stem cells from trabecular meshwork become phagocytic TM cells. *Invest Ophthalmol Vis Sci*. 2012; 53:1566–1575.
20. Du Y, Yun H, Yang E, Schuman JS. Stem cells from trabecular meshwork home to TM tissue in vivo. *Invest Ophthalmol Vis Sci*. 2013;54:1450–1459.
21. Manuguerra-Gagne R, Boulos PR, Ammar A, et al. Transplantation of mesenchymal stem cells promotes tissue regeneration in a glaucoma model through laser-induced paracrine factor secretion and progenitor cell recruitment. *Stem Cells*. 2013;31:1136–1148.
22. Ding QJ, Zhu W, Cook AC, Anfinson KR, Tucker BA, Kuehn MH. Induction of trabecular meshwork cells from induced pluripotent stem cells. *Invest Ophthalmol Vis Sci*. 2014;55: 7065–7072.
23. Ji JZ, Elyaman W, Yip HK, et al. CNTF promotes survival of retinal ganglion cells after induction of ocular hypertension in rats: the possible involvement of STAT3 pathway. *Eur J Neurosci*. 2004;19:265–272.
24. 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;198:54–64.
25. 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.
26. Pease ME, Zack DJ, Berlinicke C, et al. Effect of CNTF on retinal ganglion cell survival in experimental glaucoma. *Invest Ophthalmol Vis Sci*. 2009;50:2194–2200.
27. Mead B, Logan A, Berry M, Leadbeater W, Scheven BA. Paracrine-mediated neuroprotection and neuritogenesis of axotomised retinal ganglion cells by human dental pulp stem cells: comparison with human bone marrow and adipose-derived mesenchymal stem cells. *PLoS One*. 2014;9:e109305.
28. Ma J, Guo C, Guo C, et al. Transplantation of human neural progenitor cells expressing IGF-1 enhances retinal ganglion cell survival. *PLoS One*. 2015;10:e0125695.
29. Welsbie DS, Yang Z, Ge Y, et al. Functional genomic screening identifies dual leucine zipper kinase as a key mediator of retinal ganglion cell death. *Proc Natl Acad Sci U S A*. 2013; 110:4045–4050.
30. Davis DM, Dyer MA. Retinal progenitor cells, differentiation, and barriers to cell cycle reentry. *Curr Top Dev Biol*. 2010;93: 175–188.
31. Cepko C. Intrinsically different retinal progenitor cells produce specific types of progeny. *Nat Rev Neurosci*. 2014; 15:615–627.
32. Gill KP, Hewitt AW, Davidson KC, Pebay A, Wong RC. Methods of retinal ganglion cell differentiation from pluripotent stem cells. *Transl Vis Sci Technol*. 2014;3(4):7.
33. Young RW. Cell differentiation in the retina of the mouse. *Anat Rec*. 1985;212:199–205.
34. Marquardt T, Ashery-Padan R, Andrejewski N, Scardigli R, Guillemot F, Gruss P. Pax6 is required for the multipotent state of retinal progenitor cells. *Cell*. 2001;105:43–55.
35. Wang SW, Kim BS, Ding K, et al. Requirement for math5 in the development of retinal ganglion cells. *Genes Dev*. 2001;15:24–29.
36. Gan L, Xiang M, Zhou L, Wagner DS, Klein WH, Nathans J. POU domain factor Brn-3b is required for the development of a large set of retinal ganglion cells. *Proc Natl Acad Sci U S A*. 1996;93:3920–3925.
37. Patel A, McFarlane S. Overexpression of FGF-2 alters cell fate specification in the developing retina of Xenopus laevis. *Dev Biol*. 2000;222:170–180.
38. Meyer-Franke A, Kaplan MR, Pfrieger FW, Barres BA. Characterization of the signaling interactions that promote the survival and growth of developing retinal ganglion cells in culture. *Neuron*. 1995;15:805–819.
39. Lan L, Vitobello A, Bertacchi M, et al. Noggin elicits retinal fate in Xenopus animal cap embryonic stem cells. *Stem Cells*. 2009;27:2146–2152.
40. Ouchi Y, Tabata Y, Arai K, Watanabe S. Negative regulation of retinal-neurite extension by beta-catenin signaling pathway. *J Cell Sci*. 2005;118:4473–4483.
41. Sakuma R, Ohnishi Yi Y, Meno C, et al. Inhibition of Nodal signalling by Lefty mediated through interaction with common receptors and efficient diffusion. *Genes Cells*. 2002;7:401–412.
42. Boucherie C, Mukherjee S, Henckaerts E, Thrasher AJ, Sowden JC, Ali RR. Brief report: self-organizing neuroepithelium from human pluripotent stem cells facilitates derivation of photoreceptors. *Stem Cells*. 2013;31:408–414.
43. Tucker BA, Anfinson KR, Mullins RF, Stone EM, Young MJ. Use of a synthetic xeno-free culture substrate for induced pluripotent stem cell induction and retinal differentiation. *Stem Cells Transl Med*. 2013;2:16–24.
44. Lamba DA, Karl MO, Ware CB, Reh TA. Efficient generation of retinal progenitor cells from human embryonic stem cells. *Proc Natl Acad Sci U S A*. 2006;103:12769–12774.
45. Riazifar H, Jia Y, Chen J, Lynch G, Huang T. Chemically induced specification of retinal ganglion cells from human embryonic and induced pluripotent stem cells. *Stem Cells Transl Med*. 2014;3:424–432.
46. Xie BB, Zhang XM, Hashimoto T, et al. Differentiation of retinal ganglion cells and photoreceptor precursors from mouse induced pluripotent stem cells carrying an Atoh7/Math5 lineage reporter. *PLoS One*. 2014;9:e112175.
47. Maekawa Y, Onishi A, Matsushita K, et al. Optimized culture system to induce neurite outgrowth from retinal ganglion cells in three-dimensional retinal aggregates differentiated from mouse and human embryonic stem cells [published online ahead of print]. *Curr Eye Res*. doi:10.3109/02713683.2015.1038359.
48. Nakano T, Ando S, Takata N, et al. Self-formation of optic cups and storable stratified neural retina from human ESCs. *Cell Stem Cell*. 2012;10:771–785.
49. Eiraku M, Takata N, Ishibashi H, et al. Self-organizing optic-cup morphogenesis in three-dimensional culture. *Nature*. 2011; 472:51–56.
50. Sluch VM, Chung-ha O, Davis, Ranganathan V, et al. Differentiation of human ESCs to retinal ganglion cells using a CRISPR engineered reporter cell line. *Sci Rep*. 2015;5:16595.
51. Amoroso MW, Croft GF, Williams DJ, et al. Accelerated high-yield generation of limb-innervating motor neurons from human stem cells. *J Neurosci*. 2013;33:574–586.
52. Chambers SM, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat Biotechnol*. 2009;27:275–280.

Investigative Ophthalmology & Visual Science

53. Lu B, Malcuit C, Wang S, et al. Long-term safety and function of RPE from human embryonic stem cells in preclinical models of macular degeneration. *Stem Cells*. 2009;27:2126–2135.
54. Westenskow PD, Kurihara T, Friedlander M. Utilizing stem cell-derived RPE cells as a therapeutic intervention for age-related macular degeneration. *Adv Exp Med Biol*. 2014;801:323–329.
55. Sugita S. Retinal regeneration with iPS cells - clinical trials for retinal degenerative disorders. *Nihon Rinsho Meneki Gakkai Kaishi*. 2015;38:79–85.
56. Jha BS, Bharti K. Regenerating retinal pigment epithelial cells to cure blindness: a road towards personalized artificial tissue. *Curr Stem Cell Rep*. 2015;1:79–91.
57. Gonzalez-Cordero A, West EL, Pearson RA, et al. Photoreceptor precursors derived from three-dimensional embryonic stem cell cultures integrate and mature within adult degenerate retina. *Nat Biotechnol*. 2013;31:741–747.
58. Tucker BA, Park IH, Qi SD, et al. Transplantation of adult mouse iPS cell-derived photoreceptor precursors restores retinal structure and function in degenerative mice. *PLoS One*. 2011;6:e18992.
59. Lakowski J, Gonzalez-Cordero A, West EL, et al. Transplantation of photoreceptor precursors isolated via a cell surface biomarker panel from embryonic stem cell-derived self-forming retina. *Stem Cells*. 2015;33:2469–2482.
60. Haupt C, Huber AB. How axons see their way–axonal guidance in the visual system. *Front Biosci*. 2008;13:3136–3149.
61. Huber AB, Kolodkin AL, Ginty DD, Cloutier JF. Signaling at the growth cone: ligand-receptor complexes and the control of axon growth and guidance. *Annu Rev Neurosci*. 2003;26:509–563.
62. Huberman AD, Feller MB, Chapman B. Mechanisms underlying development of visual maps and receptive fields. *Annu Rev Neurosci*. 2008;31:479–509.
63. Koeberle PD, Bahr M. Growth and guidance cues for regenerating axons: where have they gone? *J Neurobiol*. 2004;59:162–180.
64. de Lima S, Koriyama Y, Kurimoto T, et al. Full-length axon regeneration in the adult mouse optic nerve and partial recovery of simple visual behaviors. *Proc Natl Acad Sci U S A*. 2012;109:9149–9154.
65. Omura T, Omura K, Tedeschi A, et al. Robust axonal regeneration occurs in the injured CAST/Ei mouse CNS. *Neuron*. 2015;86:1215–1227.
66. Belin S, Nawabi H, Wang C, et al. Injury-induced decline of intrinsic regenerative ability revealed by quantitative proteomics. *Neuron*. 2015;86:1000–1014.
67. Soto I, Howell GR. The complex role of neuroinflammation in glaucoma. *Cold Spring Harb Perspect Med*. 2014;4:a017269.
68. Becker CG, Becker T. Growth and pathfinding of regenerating axons in the optic projection of adult fish. *J Neurosci Res*. 2007;85:2793–2799.
69. Kador KE, Alsehli HS, Zindell AN, et al. Retinal ganglion cell polarization using immobilized guidance cues on a tissue-engineered scaffold. *Acta Biomater*. 2014;10:4939–4946.
70. Zhang N, Yan H, Wen X. Tissue-engineering approaches for axonal guidance. *Brain Res Brain Res Rev*. 2005;49:48–64.
71. Cho JH, Mao CA, Klein WH. Adult mice transplanted with embryonic retinal progenitor cells: new approach for repairing damaged optic nerves. *Mol Vis*. 2012;18:2658–2672.
72. Luo YH, Zhong JJ, da Cruz L. The use of Argus(R) II retinal prosthesis by blind subjects to achieve localisation and prehension of objects in 3-dimensional space. *Graefes Arch Clin Exp Ophthalmol*. 2015;253:1907–1914.
73. Luo YH, da Cruz L. A review and update on the current status of retinal prostheses (bionic eye). *Br Med Bull*. 2014;109:31–44.
74. Pezaris JS, Reid RC. Demonstration of artificial visual percepts generated through thalamic microstimulation. *Proc Natl Acad Sci U S A*. 2007;104:7670–7675.
75. 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;41:764–774.
76. Quigley HA, McKinnon SJ, Zack DJ, et al. Retrograde axonal transport of BDNF in retinal ganglion cells is blocked by acute IOP elevation in rats. *Invest Ophthalmol Vis Sci*. 2000;41:3460–3466.
77. Johnson EC, Deppmeier LM, Wentzien SK, Hsu I, Morrison JC. Chronology of optic nerve head and retinal responses to elevated intraocular pressure. *Invest Ophthalmol Vis Sci*. 2000;41:431–442.
78. Kermer P, Klocker N, Labes M, Bahr M. Insulin-like growth factor-I protects axotomized rat retinal ganglion cells from secondary death via PI3-K-dependent Akt phosphorylation and inhibition of caspase-3 In vivo. *J Neurosci*. 2000;20:2–8.
79. 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;305:139–142.
80. Harper MM, Grozdanic SD, Blits B, et al. Transplantation of BDNF-secreting mesenchymal stem cells provides neuroprotection in chronically hypertensive rat eyes. *Invest Ophthalmol Vis Sci*. 2011;52:4506–4515.
81. Chen G, Ma J, Shatos MA, Chen H, Cyr D, Lashkari K. Application of human persistent fetal vasculature neural progenitors for transplantation in the inner retina. *Cell Transplant*. 2012;21:2621–2634.
82. Sluch VM, Zack DJ. Stem cells, retinal ganglion cells and glaucoma. *Dev Ophthalmol*. 2014;53:111–121.
83. Tao W, Wen R, Goddard MB, et al. Encapsulated cell-based delivery of CNTF reduces photoreceptor degeneration in animal models of retinitis pigmentosa. *Invest Ophthalmol Vis Sci*. 2002;43:3292–3298.
84. 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 U S A*. 2006;103:3896–3901.
85. Goldberg JL. NT-501 CNTF Implant for Glaucoma: Safety, Neuroprotection and Neuroenhancement. Available at: https://clinicaltrials.gov/ct2/show/NCT01408472. Accessed September 15, 2015.
86. Fan BJ, Wang DY, Lam DS, Pang CP. Gene mapping for primary open angle glaucoma. *Clin Biochem*. 2006;39:249–258.

Investigative Ophthalmology & Visual Science