SPECIAL ARTICLE

Stem Cell Therapy for Ocular Disorders

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Cell injury or degeneration occurs in a number of blinding diseases. Therapy has classically consisted of preventing the initial injury or increasing the resistance of cells to injury (cytoprotection). Recently, it has become possible to repopulate tissue compartments with stem cells. This article presents a current summary of ocular stem cell research and applications to disease. It is based on presentations and discussions from the July 2002 international conference "Stem Cells and Glaucoma" sponsored by the Glaucoma Foundation. This meeting, the first of its kind, brought together ophthalmologists, geneticists, immunologists, and developmental biologists working on stem cell development and applications in both human and animal models. *Arch Ophthalmol. 2004;122:621-627*

Stem cells are undifferentiated cells able to divide indefinitely yet maintain the ability to differentiate into specific cell types. They are able to survive throughout the lifetime of the organism, while maintaining their number, producing populations of daughter cells (transit amplifying cells) that can proceed down unique pathways of differentiation. Stem cells may be obtained from embryonic tissues, umbilical cord blood, and some differentiated adult tissues. Although the potential for stem cell–based therapies for a variety of human diseases is promising, numerous problems remain to be overcome, such as methods for obtaining, transplanting, inducing differentiation, developing function, and eliminating immune reactions.[1] Stem cells have great potential value in treating eye diseases characterized by irreversible loss of cells, such as glaucoma and photoreceptor degeneration.

Although stem cells offer great opportunities for repair of the nervous system and the eye, their clinical use necessitates that we first gain an understanding of their proliferation, migration, differentiation, immunogenicity, and establishment of functional cell contacts.[2] It will also be necessary to produce these cells in conditions that meet appropriate safety and effectiveness standards. Our current understanding of the critical factors affecting stem cell behavior remains limited. Rapid progress is being made, and some of the first applications of stem cells to wound repair in human eyes have produced successes that offer hope for the use of stem cells in other ophthalmologic conditions. In this article, we discuss current concepts in stem cells and the eye and evaluate stem cell therapy in glaucoma as a paradigm for novel approaches to the treatment of eye disease.

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SOURCES OF STEM CELLS

The best understood stem cells are embryonic stem cells, which derive from early fetal development. To our knowledge, human embryonic stem cells were first characterized in 1998.[3] These cells are pluripotent (able to differentiate into a wide variety of cell types) and relatively easy to maintain in culture, but they are necessarily allogeneic (from a different genetic donor) to the potential recipient. Embryonic stem cells are continuous cell lines

and have the potential to differentiate into retinal neurons, such as photoreceptors, so they might serve as an inexhaustible source of neural progenitors for stem cell therapy in the retina. Adult stem cells, as the name implies, are derived from mature organisms and are present only in restricted cellular compartments.[4] They are multipotent (able to differentiate into a restricted number of cell types). Stem cells derived from the central nervous system (CNS) and ocular tissues have been identified as sources for cells that may someday be used to repair damaged brain, spinal cord, and retina. Stem cells within the eye have received attention because of the possibility that they could be obtained from a patient with eye disease and used autologously.

Retinal Stem Cells

Stem cells have been discovered at the pigmented ciliary margin of the adult mouse retina.[5] A mouse eye contains about 100 of these cells, while the human eye contains about 10 000. They can be isolated from eye bank eyes, even from elderly patients. Retinal stem cells do not differentiate to form brain cells yet are capable of producing all of the different retinal cell types. Although human brain stem cells grow slowly, retinal stem cells require no growth factors and grow easily and rapidly, even in completely defined serum-free media. Retinal stem cells can also be isolated from fetal retinas.[6] Both types of retinal stem cells could lead the way for stem cell ocular therapies, such as implanting photoreceptors grown in culture into the blind eye of an individual with retinitis pigmentosa or other retinal degenerative disorders.

Anterior Segment Stem Cells

Limbal stem cells located in the basal limbal area are involved in renewal of the corneal epithelium. Deficiency due to aniridia, chemical burns, Stevens-Johnson syndrome, or pemphigoid leads to conjunctivalization, neovascularization, scarring, and ulceration of the cornea. Limbal stem cells can be transplanted by using autografts in cases of unilateral disease or allografts from relatives or cadaver eyes for bilateral disease. Recently, cultured limbal stem cells have been used; a small biopsy specimen from a healthy limbus can be expanded ex vivo and then grafted to an eye with stem cell deficiency.[7] Systemic immunosuppression is necessary in all cases in which allograft limbal stem cells are transplanted, although some patients may eventually achieve a state of immunologic tolerance and immunosuppression can be discontinued. Future studies will focus on the potential use of adult pluripotent stem cells for ocular surface reconstruction and also strategies for promoting a state of tolerance in allograft limbal transplantation.[8,9]

Autologous conjunctival biopsy specimens obtained in the superior fornix, where conjunctival stem cells reside, can be expanded in tissue culture by using amniotic membrane as a carrier and can then be surgically transplanted to the ocular surface. These tissue equivalents have been used successfully in conjunctival replacement after pterygium surgery and for repair of leaking from a scarred filtering bleb. This use of conjunctival stem cells suggests additional future applications for these tissue equivalents.

The corneal endothelium may contain regions for storage (most peripheral), regeneration (paracentral), and migration of stem cells. An area of corneal endothelial cells adjacent to the Schwalbe line may be able to transit amplifying cells and slow-cycling cells.[10] Endothelial cell density is markedly increased in this area, as compared with central endothelial cell density.[11,12]

Although cell division in the normal primate trabecular meshwork is rare, a niche for trabecular meshwork stem cells might exist at the Schwalbe line in monkeys. Cells at the Schwalbe line appear different from trabecular meshwork cells, and cells with a similar phenotype to the former seem to migrate to the trabecular meshwork.[13,14] Transplantation of trabecular meshwork stem cells to glaucomatous eyes might improve aqueous outflow. Theoretically, trabecular meshwork stem cells might be isolated from adult or embryonic trabecular meshwork. Embryonic stem cells are available, but there is a substantial risk that if transplanted into the chamber angle, they will not differentiate but rather continue to proliferate in the chamber angle. This would worsen, rather than improve, outflow. It is therefore necessary to identify mechanisms to induce differentiation of embryonic stem cells to cells that express the trabecular meshwork phenotype.

DIFFERENTIATION OF STEM CELLS

To obtain large numbers of engrafted stem cells that differentiate in a desired way, strategies are needed to channel cells into desired phenotypes.[15] Modification of the microenvironment and/or inhibition of intracellular signaling cascades in engrafted cells will be needed for appropriate cell-specific differentiation into injured tissue.[16]

There are several likely sources of neural progenitors with retinal potential that may make stem cell therapy for eye disease possible.[17] Progenitors isolated from later stages of retinal development, which normally do not give rise to retinal ganglion cells (RGCs), can develop into RGCs in conducive conditions.[18] These cells extend processes to tectal explants (a target for the RGC axon) and appear to function like RGCs. Stem cells derived from adult pigmented ciliary epithelium are an excellent source of retinal progenitors because they can differentiate along photoreceptors and RGC lineages.[19] Another readily accessible and promising source of neural progenitors for autologous stem cell therapy is the adult limbal epithelium (also discussed in the earlier subsection on anterior segment stem cells). Although nonneural in origin, progenitors from limbal epithelium can generate both neurons and glia.[20]

The influence of the age of the host on the fate of stem cells after transplantation has been studied in the Brazilian opossum, a small pouchless marsupial whose young are born in an immature state.[21] Brain progenitor cells from mice expressing green fluorescent protein as

a marker were transplanted via intraocular injection into developing and mature opossum eyes.[22] These cells differentiated in host retinas, often displaying morphologies characteristic of RGCs, amacrine cells, bipolar cells, and horizontal cells. Transplanted cells generally followed the architectural organization of the host eyes. The greatest morphological integration and differentiation was observed in the youngest host eyes, with little integration in mature eyes. Transplanted brain progenitor cells may be capable of responding to local microenvironmental cues that promote their differentiation and integration.

Gene expression analysis can be used to determine the genes involved in the transition from the multipotent to the differentiated state. New retinal cells are continually added at the ciliary marginal zone in fish and amphibians,[23] and it is possible to study the underlying molecular mechanisms of stem cells by comparing gene expression profiles between undifferentiated and differentiated states or between multipotent and nonmultipotent states.[24-26]

STEM CELLS IN INJURED EYES

Tissue injury provides a host of factors that influence the fate of implanted stem cells and restricts their terminal lineages.[16] For example, adult rat hippocampal neuronal progenitor cells have been transplanted into the vitreous of glaucomatous rats in the hope that they would repopulate the retina as RGCs. Some of the progenitor cells injected into the vitreous expressed the neuronal tissue-specific microtubule-associated protein 2, which suggests that they start to develop into a neuronal phenotype (D. S. Sakaguchi, PhD, et al, unpublished data, 2003).

Adult human neural progenitor cells grafted to diseased hosts can express mature neuronal markers, send processes to the appropriate plexiform layer, and extend neurites into the optic nerve.[27] In specific developmental and injury conditions, brain-derived cells can differentiate into cells similar to retinal neurons. However, in the mature (postmitotic) retina, this transformation is difficult to achieve. Local microenvironmental cues influence phenotypic differentiation of grafted cells. Retinal stem cells derived from mice expressing green fluorescent protein can develop into photoreceptors and bipolar cells in vitro and in vivo.[28] With the development of tissue engineering, retinal stem cells impregnated into polymers might be grafted into the subretinal space.

Neural stem cell spheres have been injected into the vitreous of DBA/2J mice, which have hereditary pigmentary glaucoma (M. J. Young, PhD, et al, unpublished data, 2003). In 14-month-old mice with mild depletion of their RGCs, some transplanted cells entered the retina, elaborated processes, expressed neurofilaments, and actually sent processes into the plexiform layer. At 4 months after transplantation, fibers were seen entering the optic nerve head.

STEM CELLS IN LOWER ANIMALS

Studies of stem cells in lower animals may provide insights to their application in mammals. The *Drosophila melanogaster* ovary provides an attractive model to study stem cell biology because both stem cells and their surrounding cells have been well defined.[29] Many stem cell properties and relationships to their microenvironments, or niches, can be effectively studied at molecular and cellular levels. This *Drosophila* system revealed critical issues in stem cell research, including the importance of the niche within which the stem cells and daughter cells differentiate or remain unchanged, the ability to identify individual gene products of importance to differentiation status, and the opportunities to use bioinformatics to apply findings from a model to the study of humans.

As a fish grows, so does its CNS, including the retina. Retinal growth is partly due to the continual generation of new neurons. Furthermore, unlike injury in human nervous tissue, injury to the fish retina is repaired by regenerative neurogenesis.[30] Persistent and injury-induced neurogenesis in the fish retina is due to the presence of stem cells that perpetually reside in this tissue. Studies are under way to identify the genes expressed by retinal stem cells and the molecules that regulate their neurogenic activity, both during normal growth and after injury.[31,32] Knowledge of how these cells maintain their ability for neurogenesis may eventually be applicable to human cells.

STEM CELLS AND GLAUCOMA

The only treatment proved for glaucoma is pharmacological or surgical lowering of the intraocular pressure, but disease in many patients progresses despite treatment. Even if new treatments were developed that could stop all future development of visual field loss, there would still be a substantial need to deal with the profound visual field losses in millions of people who have had glaucoma in the past. Neuroprotection of RGCs and their axons[33] is an alternate treatment being investigated in randomized controlled trials.[34] Other potential treatments for glaucoma are vaccination with antigens that can induce protective autoimmunity[35] and improvement of ocular blood flow.[36]

There are at least 3 potential targets for stem cell therapy in glaucoma: the RGC, the optic nerve head, and the trabecular meshwork. So far, most work has focused on replacing RGCs because their death is the final common pathway for visual loss in glaucoma and other optic neuropathies. Because human RGCs are mammalian CNS neurons that cannot divide and differentiate to replace other cells lost from disease, blindness from glaucoma is irreversible. Finding a way to differentiate stem cells into RGCs and allow them to connect to their appropriate targets would be a major step in repopulating the neurons lost in glaucoma. The main issues to be resolved are survival and differentiation of the stem cell, maintaining the state of the surrounding microenvironment, extension of axons into the optic nerve, establishment of functional connections in the lateral geniculate nucleus, and appropriate activation of transsynaptically connected cortical targets.

The RGC precursor cells introduced into the retina extend processes into the optic nerve head.[27] The need to establish a functional network communicating information to the brain makes the problem of stem cell replacement of RGCs especially complex. However, because patients lose a substantial portion of their RGCs before developing functional deficits, there is hope that a limited amount of restoration might have a large effect on visual capability.

Efforts to repair the trabecular meshwork could theoretically improve intraocular pressure regulation. Restoration of the entire trabecular meshwork might not be needed, and simple replacement of corneoscleral cells might suffice because juxtacanalicular cells are not depleted in late stages of glaucoma.[37] Although eye bank eyes might constitute a plentiful source of trabecular meshwork cells, it remains to be seen whether rejection of allogeneic cells would be a problem.

A third target for cellular repletion is the optic nerve head, which undergoes substantial remodeling and biochemical change in glaucoma. Issues to be dealt with in the region of the optic nerve head include excavation, activation of astrocytes, secretion of nitric oxide, vascular complications, and loss of cellularity.[38-40] Progression of glaucomatous optic neuropathy in the presence of what appears to be clinically adequate lowering of intraocular pressure may reflect structural and functional changes of optic nerve head cells. Repopulating these cells with stem cell–derived normal astrocytes and fibroblasts might be an alternate therapy for glaucoma—one that does not require complex axonal pathfinding.

The optimal source of stem cells for a particular therapy is a major issue. Not all stem cell types can be induced to differentiate into all of the cell types needed to treat glaucoma. It is encouraging that a number of different sources and types of stem cells and precursor cells have been identified from which relevant cell types for ocular stem cell therapy can be derived. These include not only fetal stem cells but also cells from brain, limbus, conjunctiva, corneal endothelium, and retina. One question that has not yet been addressed is whether stem cells are needed at all, or whether at least some problems in either the optic nerve head or the trabecular meshwork could be solved by simply transplanting young healthy differentiated cells into the damaged eye.

Another approach to improving the local environment could come through genetic modification of cells before they are introduced. Successful use of such approaches will require more work on determining which genetic modifications are needed and also on overcoming problems with gene silencing that can happen not only after differentiation but also in some cases in the course of passaging cells as they are being grown for use. In the end, it seems likely that some type of "nurse" cells that clean up the environment or provide supporting factors will need to precede or accompany the primary cells being restored. In addition, protection of the fragile new cells may also arise from work on a vaccination approach to recruitment of cells from the immune system with neuroprotective functions.

CHALLENGES IN USING STEM CELLS FOR EYE DISEASE

Safety

There are strict rules for the conditions in which stem cells must be grown if they are destined for therapeutic use. Cells must be grown without serum and without the use of cell feeder layers, something that would potentially complicate maintenance of at least some stem cell types that require other cell types in their local niche to maintain an intermediate state of differentiation. With the substantial amount of work required for approval to use a given cell line, it will be essential that such cells can be grown in large amounts while maintaining a particular stage of multipotent development. In addition, the cells must differentiate in completely defined conditions, their proliferation after transplantation must be shut down, and they must perform the desired functions while remaining localized to their site of targeting.

Microenvironment of the Transplanted Stem Cell

Another critical issue is that of the environment into which replacement cells will be introduced. The processes by which stem cells settle, differentiate, and extend axons in an adult eye do not recapitulate what happens during development, and the environment into which they are introduced may be hostile, as compared with the environment in which the original developmental processes took place. Restricted ability of neural implants to survive, migrate, and reestablish neuronal connections with the host environment has limited the success of neural transplantation. It is unclear whether the postnatal trophic support is sufficient to maintain stem cell survival[41] or whether scaffolding such as glial cells or extracellular matrix will adequately protect the new axons that are attempting to make connections and transmit impulses. Pretreatment of the injured tissue with growth factors such as neurotrophins or repletion of supporting cells such as astrocytes or other cells producing trophic or differentiating factors could greatly assist the survival of the new cells in a previously hostile environment. The ability of transplanted neural graft cells to migrate and integrate into the host retina can be influenced by intrinsic properties of implant cells, as well as by factors in the host retinal environment.

Continued Progression of Disease

Not only will the differentiated stem cell face a hostile environment after transplantation, but it also may be just as susceptible to the disease as the original cell. For example, a repopulated RGC in an eye with advanced glaucoma may not survive because of the underlying pathophysiology of the disease. If it took many years for the adverse environment to kill the RGCs, the newly transplanted RGCs might survive longer, and the restoration of optic nerve function could last many years. If not, the restored cells might soon die unless steps are first taken to correct the environment,

which is a more complex problem. Thus, the problem of preexisting injury in a diseased eye necessitates that the damage from the surrounding environment be repaired in addition to replacing the primary cells of interest.

Pathfinding

The optic nerve carries axons from RGCs to targets in the lateral geniculate nucleus in a retinotopic mapping. For the injured optic nerve to be restored, there has to be pathfinding of transplanted retinal stem cells within the retina that migrate to the appropriate cellular location and send axons to the optic nerve head, then through the nerve, 50% crossing at the chiasm, and eventually to a correct location in the lateral geniculate nucleus.[42,43] Retinal glial cells, including astrocytes and Müller cells, are the guardians of the retinal cell layers. In the host retina, they play an essential role in preventing graft cell migration and integration. It may be possible to guide the migration of transplanted neural stem cells by selectively manipulating glial cell properties in the host retinal environment. Ensuring crossing at the chiasm and precise arrival at axonal targets remains a difficult problem.[44,45]

Remyelination

Not only is it necessary for RGCs to reach their appropriate targets, but physiological axonal conduction velocity and energy efficiency also require the presence of myelin. Our knowledge of remyelination in adult human CNS derives from the extent, or lack thereof, of remyelination observed in multiple sclerosis. This observation raises a number of issues regarding the potential role of progenitor cells in replacing injured myelin and oligodendrocytes in adult human CNS. Remyelination occurs in acute rather than chronic multiple sclerosis lesions. Oligodendrocyte progenitor cells can be identified in regions of active multiple sclerosis lesions but without apparent increase in numbers, as compared with findings in normal brain tissue.[46] At issue is whether the remyelinating cells are derived from a pool of glial-restricted progenitor cells or from multipotent stem cells that migrate from subependymal regions. The apparent failure of ongoing remyelination in multiple sclerosis could reflect a number of factors, including exhaustion of progenitor cells, lack of trophic signals, injured axons being unreceptive to remyelination, and selective immune injury of the progenitors.[47,48] These issues will need to be understood for effective remyelination of the optic nerve.

Immune Response

Allogeneic stem cells are potential targets for the immune system, and their use may be hindered by humoral or cell-mediated rejection. This may be tempered by the relatively immune-privileged nature of the eye. Although the existence of stem cells in the adult eye or other organs offers the possibility of bypassing allogeneic rejection through use of the patient's own stem cells, such cells might carry whatever defect initially predisposed the eye to glaucoma and therefore fail to correct the problem.

FUTURE DIRECTIONS

There is ample precedent for research in stem cell therapy for neurodegenerative disease. A good example is motor neuron disease. In animal models of amyotrophic lateral sclerosis, human embryonic brain–derived cells produce rapid and profuse motor neuron growth when engrafted into the ventral horn of the spinal cord. Transplanted embryonic brain–derived cells acquire immunohistochemical markers of mature neurons and astrocytes and send axonal processes to the periphery. This process allows paralyzed animals to move their hind limbs and walk.

The same will likely be true for using stem cells for ophthalmic disease. Precursor cells can settle in the retina, differentiate into RGCs, connect with afferent neurons in the inner plexiform layer, and grow into the optic nerve head. Many details need to be worked out about the use of such cells to restore the complex delicate neural network of the eye. Not only cellular regrowth but also establishment of functional connections to the lateral geniculate body will be required.

There are at least 5 different areas in which progress needs to be achieved. First, more needs to be known about precursor cells for any given cell type and about their production of essential factors, which may mean identification of stem cells that can differentiate into cell types that can live (eg, in the optic nerve head) and produce the appropriate factors. Alternatively, it may mean genetic modification of cells to achieve expression of genes encoding such factors. Desired functions include interfering with apoptosis, inhibiting factors in amacrine cells, and production of trophic factors.

Second, much more needs to be known about potential sources of stem cells that could be developed in appropriate conditions and that could be approved for therapy. This includes the need to explore embryonic stem cells, brain stem cells, ocular stem cells, and even the transplantation of some types of mature differentiated cells.

Third, microenvironments need to be identified in which stem cells can proliferate; differentiate; engraft; migrate; and, once they are in the right state and place, shut down proliferation. This may include the need to understand more about other cell types in the surrounding environments and on the topographic map of the target region.

Fourth, study of naturally regenerating systems such as teleost or chick eye offer the chance to identify antagonists for inhibitors of regeneration in the retina and to identify the genes involved in growth and differentiation early in development, when the ability of the mammalian retina to regenerate has not yet been shut down.

Fifth, even some of the simplest experiments on transplantation of trabecular meshwork cells have yet to be performed, and little is known about any potential stem cells or precursor cells from which the trabecular meshwork might be revived.

SUMMARY

Although preliminary results have been achieved on many different fronts in applying stem cell technol-

Glaucoma Foundation Meeting Attendees

The Glaucoma Foundation held a meeting entitled "Stem Cells and Glaucoma" in Chicago, Ill, July 26-27, 2002. The meeting organizers and moderators were Terete Borrás, PhD (University of North Carolina at Chapel Hill, Chapel Hill, NC), Leonard A. Levin, MD, PhD (University of Wisconsin, Madison, Wis), Julia E. Richards, PhD (University of Michigan, Ann Arbor, Mich), and Robert Ritch, MD (New York Eye and Ear Infirmary, New York, NY). Participants included J. Wayne Streilein, MD (President, Schepens Eye Research Institute, Boston, Mass), Theodore Krupin, MD (Clinical Professor of Ophthalmology, Northwestern University School of Medicine, Evanston, Ill), Derek van der Kooy, PhD (Professor of Anatomy and Cell Biology, Faculty of Medicine, University of Toronto, Ontario, Canada), Scott Whittemore, PhD (Professor of Neurological Surgery, University of Louisville School of Medicine, Louisville, Ky), Ryo Kubota, MD, PhD (Assistant Professor, Department of Ophthalmology, University of Washington, Seattle), Young Kwon, MD, PhD (Associate Professor of Clinical Ophthalmology, Department of Ophthalmology, University of Iowa, Iowa City), Michael J. Young, PhD (Director, Minda de Gunzburg Research Center for Retinal Transplantation, Harvard Medical School, Boston, Mass), Simon John, PhD (Associate Investigator, Jackson Laboratories, Howard Hughes Medical Institute, Bar Harbor, Me), Ting Xie, PhD (Assistant Scientist, Stowers Institute for Medical Research, Kansas City, Mo), Peter Hitchcock, PhD (Associate Professor of Ophthalmology and Visual Sciences and Developmental Biology, University of Michigan, Ann Arbor, Mich), Iqbal Ahmad, PhD (Associate Professor of Ophthalmology and Pharmacology, University of Nebraska Medical Center, Omaha), Donald Sakaguchi, PhD (Associate Professor of Zoology and Genetics, Iowa State University, Ames, Iowa), Jeffrey Rothstein, MD, PhD (Professor of Neurology and Neuroscience, Johns Hopkins University, Baltimore, Md), Dong Feng Chen, PhD (Assistant Professor, Department of Ophthalmology, Harvard University, Boston, Mass), Henry Edelhauser, PhD (Director of Ophthalmic Research, Emory Eye Center, Atlanta, Ga), Ernst Tamm (Professor of Molecular Anatomy and Embryology, Department of Anatomy, University of Erlangen-Nürnberg, Erlangen, Germany), Jack Antel, MD (Professor of Neurology, Montreal Neurologic Institute, Quebec, Canada), Ali Djalilian, MD (National Eye Institute, Bethesda, Md), Michal Schwartz, PhD (Professor of Neuroimmunology, Weizmann Institute of Science, Rehovot, Israel), Roger Beuerman, PhD (Scientific Director, Singapore Eye Research Institute, Singapore), William W. Hauswirth, PhD (University of Florida, Gainesville), Paul Kaufman, MD (University of Wisconsin, Madison), Erin B. Lavik, ScD (Massachusetts Institute of Technology, Cambridge, Mass), James C. Tsai, MD (Columbia University, New York, NY), Abbot F. Clark, PhD (Alcon Research Ltd, Fort Worth, Tex), John Donello, PhD (Allergan Inc, Irvine, Calif), and Vincent Michael Patella, OD (Carl Zeiss Ophthalmic Systems Inc, Dublin, Calif).

ogy to eye disease, a tremendous amount of work remains. Some of the main issues are identification of the optimal precursor cell types, establishment of growth and differentiation conditions that meet safety and effectiveness standards, and the manipulation of the surrounding environment to allow transplanted cells to survive and function. Although transplanted neuronal precursors can connect into the inner plexiform layer and optic nerve head, precisely directing their axons to appropriate targets remains to be demonstrated. Substantial progress in this and other areas needs to be achieved, particularly with respect to diseases like glaucoma and other optic neuropathies, before accurate reassembly of the complex visual pathways will be achieved.

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