Current approaches and future prospects for stem cell rescue and regeneration of the retina and optic nerve

Annegret Dahlmann-Noor, MD, PhD; Sauparnika Vijay; Hari Jayaram, MD, G; Astrid Limb, PhD; Peng Tee Khaw,* MD, PhD

ABSTRACT • RÉSUMÉ

The 3 most common causes of visual impairment and legal blindness in developed countries (age-related macular degeneration, glaucoma, and diabetic retinopathy) share 1 end point: the loss of neural cells of the eye. Although recent treatment advances can slow down the progression of these conditions, many individuals still suffer irreversible loss of vision. Research is aimed at developing new treatment strategies to rescue damaged photoreceptors and retinal ganglion cells (RGC) and to replace lost cells by transplant. The neuroprotective and regenerative potential of stem and progenitor cells from a variety of sources has been explored in models of retinal disease and ganglion cell loss. Continuous intraocular delivery of neurotrophic factors via stem cells (SC) slows down photoreceptor cells and RGC loss in experimental models. Following intraocular transplantation, SC are capable of expressing proteins and of developing a morphology characteristic of photoreceptors or RGC. Recently, recovery of vision has been achieved for the first time in a rodent model of retinal dystrophy, using embryonic SC differentiated into photoreceptors prior to transplant. This indicates that clinically significant synapse formation and acquisition of the functional properties of retinal neurons, and restoration of vision, are distinct future possibilities.

Les 3 causes les plus communes de la déficience visuelle et de la cécité légale des pays développés (dégénérescence maculaire liée à l'âge, glaucome et rétinopathie diabétique) partagent un seul point final : la perte des cellules nerveuses oculaires. Bien que les progrès récents du traitement puissent ralentir la progression de ces maladies, beaucoup de personnes souffrent toujours d'une perte irréversible de vision. La recherche vise le développement de nouvelles stratégies de traitement pour sauver les photorécepteurs endommagés et les cellules ganglionnaires de la rétine (CGR) et remplacer les cellules perdues par des greffons. L'on a examiné les possibilités neuroprotectrices et régénératrices des cellules souches et progénitrices de diverses sources dans des modèles de maladie rétinienne et de perte de cellules ganglionnaires. Le dégagement intraoculaire continu de facteurs neurotrophiques par la voie des cellules souches (CS) ralentit la perte des cellules photoréceptrices ainsi que des CGR chez les modèles expérimentaux. À la suite de la greffe intraoculaire, les CS peuvent émettre des protéines et développer une morphologie caractéristique des photorécepteurs ou des CGR. Récemment, le recouvrement de la vue a été réalisé pour la première fois dans un modèle de dystrophie rétinienne de rongeur, à l'aide de CS embryonnaires différenciées en photorécepteurs avant la greffe. Cela indique que la formation de synapses cliniquement significatives et l'acquisition de propriétés fonctionnelles des neurones rétiniennes, et la restauration de la vision sont d'éventuelles possibilités distinctes.

Age-related macular degeneration (AMD), glaucoma, and diabetic retinopathy are the 3 most common causes of visual impairment and legal blindness in developed countries.[1] One common denominator of these conditions is progressive loss of the neural cells of the eye (photoreceptors, interneurons, and retinal ganglion cells [RGC]) (Fig. 1) and essential supporting cells, such as the retinal pigment epithelium (RPE). Retinal dystrophies (retinitis pigmentosa [RP], Stargardt disease, Best disease, Leber congenital amaurosis, etc.) share the early loss of photoreceptors and subsequent loss of RGC. Recent years have seen enormous progress in treatment options to stop the progression of AMD from a neovascular state to fibrosis, to slow down progression of glaucoma by reducing intraocular pressure, and to prevent progression of diabetic retinopathy by optimizing glycemic control and treating retinal neovascularization early.[2–7] However, irreversible visual loss still occurs in a significant proportion of cases. Research is aimed at developing novel treatments using neuroprotective and regenerative strategies.

Stem cells (SC) can potentially be used for both neuroprotection and cell replacement. Intravitreal delivery of neurotrophic factors slows down photoreceptor degeneration in rodent models of RP and RGC loss in glaucoma models and optic nerve (ON) and optic tract (OT) trauma, but the effect may be temporary.[8] Slow-release preparations

From *the NIHR Biomedical Research Centre, Moorfields Eye Hospital and UCL Institute of Ophthalmology, London, United Kingdom, and UCL Partners AHSC, London, United Kingdom

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Correspondence to Peng T. Khaw, MD, PhD, NIHR Biomedical Research Centre in Ophthalmology, Moorfields Eye Hospital and UCL Institute of Ophthalmology, 11-43 Bath St., London EC1V 9EL, U.K.; p.khaw@ucl.ac.uk

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and gene therapy approaches to induce retinal cells to secrete neurotrophic factors are 2 ways to induce longer-term effects.[9–15] A third option is to use SC as long-term delivery agents, possibly encapsulated in a device, because many SC either secrete neurotrophins naturally or can be genetically engineered to do so.

Progress has also been made in the field of photoreceptor, RPE, and RGC replacement by SC and progenitor cells, although long-term restoration of visual function has been exceptional. The recent discoveries that human fibroblasts can be "reprogrammed" to behave like embryonic SC (ES) and that adult eyes harbour retinal progenitor cells also increase the potential availability of SC for transplantation, including autologous transplantation and stimulated intrinsic "self-regeneration," which could potentially overcome a lot of the problems associated with nonautologous transplantation in humans.

WHAT ARE STEM AND PROGENITOR CELLS?

Ideally, cells for retinal and ON cell replacement should be widely available, easy to culture, able to migrate and integrate into the retina and ON without inducing an immunological response, and should differentiate into the desired type of retinal cell (photoreceptor, RGC) and form appropriate cellular extensions and synaptic connections to restore visual function. Additionally, it would be ideal if they had the ability to stimulate, rescue, and protect existing compromised neuronal cells. SC have the potential to meet these demands.

By definition, SC have the ability to renew their population by cell division and to differentiate into different cell types when exposed to appropriate stimuli or environmental triggers. Investigations into retinal and ON repair have used stem and progenitor cells, the difference between the 2 cell types being that SC can differentiate into any cell type (totipotent or pluripotent), whereas progenitor cells can differentiate into cells from 1 closely related family of cells (multipotent) or into 1 cell type only (unipotent) but still have the property of self-renewal.[16]

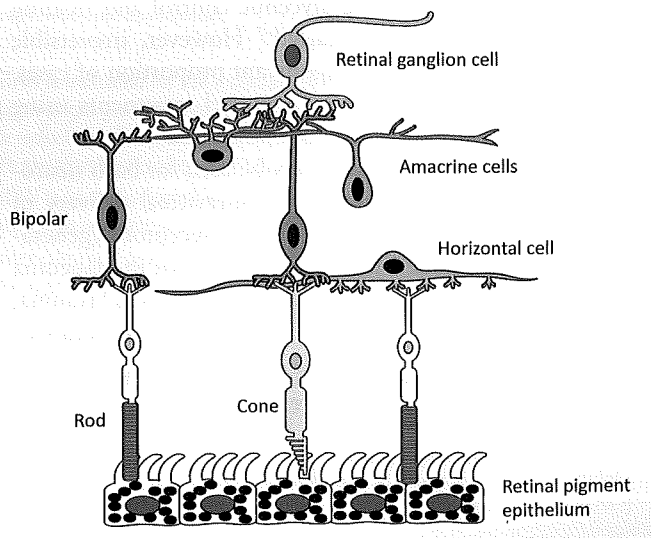

Fig. 1—Targets for cell replacement and regeneration strategies: photoreceptors, retinal ganglion cells and axons, retinal interneurons, and retinal pigment epithelium. (Figure adapted with permission from a diagram by Motifolio Inc.)

Stem and progenitor cells from different sources have been tested in models of photoreceptor, RPE, and RGC replacement (Fig. 2). SC can be categorized broadly into ES, tissue-derived SC, and induced pluripotent SC (iPS). ES are isolated from the inner cell mass of blastocysts. Adult or tissue-derived SC are cell populations surviving in niches of adult organisms. ES and iPS can differentiate into any type of cell, whereas tissue-derived SC are usually limited to the differentiation into cells specific to their location.

WHAT CAN SC DO FOR THE VISUAL PATHWAY?

To date, SC have been used most successfully in animal models of photoreceptor or RGC rescue. Following transplant into the vitreous cavity, subretinal space, ON, or OT, SC mediate temporary photoreceptor and RGC survival in models of retinal disease, glaucoma, and surgical lesions, as detailed later in this text. The main mechanism for this beneficial effect appears to be secretion of neurotrophic factors, which increase photoreceptor and RGC survival and might even facilitate RGC axon regrowth (Fig. 2). These factors include ciliary neurotrophic factor, fibroblast growth factor 2, brain-derived neurotrophic factor, and glial-derived neurotrophic factor, but there may be other factors yet to be discovered.[17–19]

In addition, many studies have investigated potential photoreceptor, RPE, and RGC replacement by SC. Although migration and integration into the retina and the expression of photoreceptor-specific and RGC-specific proteins have been observed in most published studies, synapse formation and functional recovery have been exceptionally rare.[20]

Attempts have also been made to remyelinate damaged ON axons by transplanting Schwann cells or olfactory ensheathing cells (OEC) into ON lesions.[21–24] These cells might exert neurotrophic and mechanoprotective effects and also overcome growth-inhibiting glial scars.

WHERE CAN WE GET SC FROM?

The many sources of SC are summarized in Figure 2. ES are isolated from the inner cell mass of blastocysts, a developmental stage reached in humans at 5 days after fertilization. They have been derived predominantly from animals and have been used mostly in animal studies, because the use of human ES (hES) raises ethical concerns. Although ES have the full differentiation potential, differentiation is a lengthy process and is difficult to standardize. ES for transplantation into the eye can be manipulated into expressing photoreceptor and RGC-specific proteins. Growth factors or drugs are added to culture media to simulate the retinal environment.

The addition of epidermal growth factor, basic fibroblast growth factor,[25,26] or somatostatin,[27] or coculture with embryonic retinal explants,[27] are some examples of techniques used to modify the culture microenvironment prior to transplantation. In addition to ethical problems there are concerns that ES have genomic and chromosomal instability and can give rise to teratomata and other tumours.[28–33]

Mesenchymal SC (MSC) can be derived from bone marrow (BM) and human umbilical cord blood and have the advantage of allowing autologous transplantation. Drugs such as plerixafor are available to mobilize hematopoietic SC from the BM in humans. The aim is to move SC into the bloodstream, where they can be harvested easily.[34] Strategies to mobilize other types of BM-derived SC, including MSC, such as sequential administration of vascular endothelial growth factor and a chemokine receptor (CXCR4) inhibitor, are under development.[35,36]

Neural stem and progenitor cells for ocular and central nervous system (CNS) repair have been isolated from the adult forebrain and hippocampus. Adult hippocampal precursor cells (AHPC) are derived from the dentate gyrus of the hippocampus, an area of continuous neuron renewal even in adult organisms.[27,37–41]

At present, stem and progenitor cells derived from the eye are the most successful cell type when it comes to differentiation into retina-specific cells. These cells can be isolated at different stages of development. The optimal developmental stage of donor cells for transplant is not yet clear, although 1 study[20] with functional improvement showed that the stage of transplanted cell development was critical. We do not know if it is better to induce differentiation of an undifferentiated cell in vitro, with transplantation of the differentiated cell, or to transplant an undifferentiated cell and then differentiate it into the desired cell once it is in the target microenvironment. The answer may lie in the middle, because the environmental cues in the adult retina in need of repair differ widely from those in the prenatal developing retina. However, more differentiated donor cells do not migrate or integrate well into the host retina.[20]

Retinal stem and progenitor cells have been isolated from dissociated embryonic or neonatal retina[25,26] and from the anterior retinal margin known as the "ciliary marginal zone" in adult eyes.[42,43] In addition, a subpopulation of Müller cells with SC characteristics (Moorfields/Institute of Ophthalmology or MIO cell) has been identified in adult human retina.[43,44]

It was felt previously that cells from the ciliary body had significant retinal SC potential.[45–47] However, evidence is mounting that these cells may not possess retinal SC properties.[48,49]

iPS avoids the ethical problems surrounding the use of SC. These pluripotent SC are obtained by "reprogramming" fibroblasts to behave like "genuine" ES. Since their first descriptions in mice[50] and humans,[51] the technology of obtaining iPS has become safer by avoiding the use of viral vectors.[52,53]

Another type of progenitor cell used in CNS repair studies is the OEC. These are glial cells in the nasal mucosa and the olfactory bulb that guide and ensheath the continuously regenerating axons of the olfactory nerve from the nose to their target at the base of the brain. In models of spinal cord injury, these cells support regenerating axons and restore function.[54–57]

HOW CAN WE DELIVER STEM AND PROGENITOR CELLS TO THE VISUAL SYSTEM?

A variety of transplant strategies have been used in animal models (Fig. 3). The intravitreal and subretinal routes

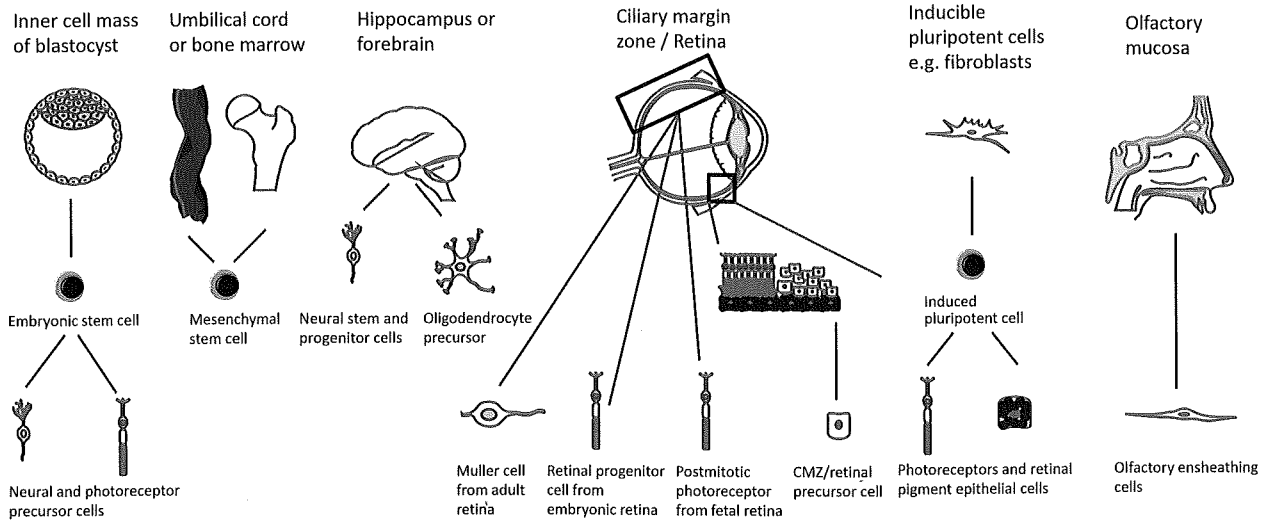

Fig. 2—Sources of stem and progenitor cells for retinal cell replacement. (CMZ, ciliary marginal zone.) (Figure composed using Motifolio Inc. diagrams.)

are used most commonly; these rely on migration of grafted cells into the retina or ON, with subsequent integration and synapse formation.

Another approach to intravitreal placement of SC is to encapsulate the cells in a polymer, resulting in a basket of cells that continuously release neurotrophic factors into the vitreous cavity. An intravitreal implant of fibroblasts engineered to secrete human fibroblast growth factor 2 and encapsulated in a polymer microcapsule slowed down photoreceptor degeneration in a rat model of RP.[58] An intravitreal implant containing cells derived originally from a human RPE cell line (ARPE-19) and genetically engineered to express ciliary neurotrophic factor has recently undergone a phase I trial in humans. In addition to demonstrating safety, this trial also recorded functional improvement in some participants.[59,60] Both these studies indicate that a continuous supply of neurotrophic factors can be achieved with current techniques and does not require SC.

Further upstream, SC can be injected into ON and OT lesions. Human umbilical cord blood–derived MSC injected into an OT lesion rescued RGC and promoted long-distance regrowth to the superior colliculus in rats.[61]

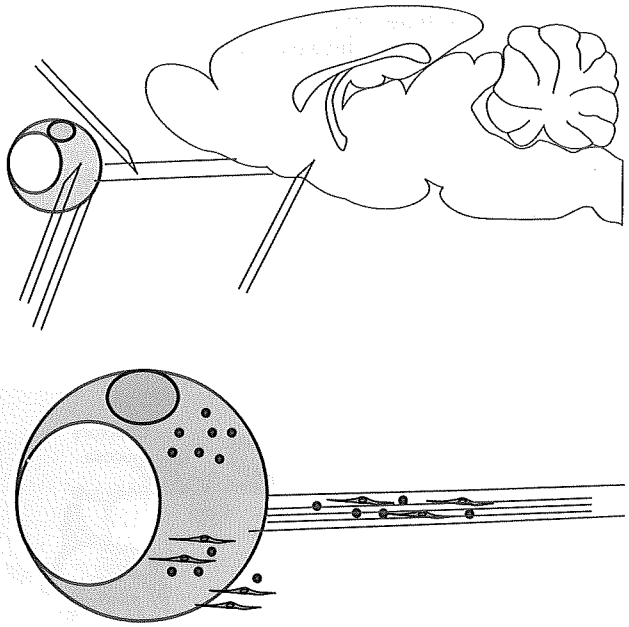

Fig. 3—Stem and progenitor cell delivery to the eye and rodent brain (top) and their mechanism of action (bottom). The most commonly used routes are intravitreal and subretinal injections. Intravitreally placed polymer capsules containing stem cells (SC) can be used as a continuous source of neurotrophic factors. SC transplanted into models of retinal dystrophy and optic nerve and optic tract lesions promote photoreceptor and retinal ganglion cells (RGC) rescue and regeneration. Olfactory ensheathing cells might provide neurotrophic and mechanoprotective support by ensheathing RGC axons. SC integrating into the retina express proteins characteristic of photoreceptors or RGC, although synapse formation and functionality remain the exception. (Figure composed using Motifolio Inc. diagrams.)

Systemic injection of BM-derived MSC slowed down photoreceptor degeneration and the development of abnormal blood vessels in a rat model of RP.[62] This indicates that these cells are capable of "homing in" from the systemic circulation onto diseased retinal tissue. Although the grafted cells integrate into the retina, their main mechanism of action might be to induce local Müller cells to secrete brain-derived neurotrophic factor.[62] However, in the first human patient, BM-MSC transplantation did not improve visual function.[63]

CAN SC REPLACE PHOTORECEPTORS?

SC therapies for photoreceptor replacement provide an exciting prospect for the restoration of sight for those whose vision has been significantly damaged by degenerative retinal diseases affecting primarily the photoreceptors for which no treatments are currently available. Replacement of a unidirectional sensory neuron, such as the photoreceptor, might be less difficult than that of RGC, which have complex afferent inputs and distant synapses.

Early research studied the localization of cells following transplant into rodent models of retinal degeneration. Compared with intravitreal injection, SC seemed to migrate and integrate more easily when transplanted beneath damaged retina.[39] The intrinsic microenvironment[64] or local intrinsic signals[65] might also influence progenitor cell fate in vivo.

Recent studies have challenged the concept that an undifferentiated cell is the ideal candidate for transplant strategies to replace photoreceptors. A landmark study defined the optimal developmental stage for the transplant of photoreceptor precursors in a mouse model of retinal degeneration, demonstrating both structural integration of transplanted cells and functional improvement with pupillometry.[20] Critically, SC were found to perform best when differentiated into postmitotic photoreceptor precursors in vitro, prior to transplantation.

To facilitate the development of human therapies, work has moved towards the development of protocols to differentiate hES in vitro to provide a potential source of cells for transplant. These studies have produced cells expressing photoreceptor-specific markers.[66,67] Transplantation into the subretinal space of a mouse model of Leber congenital amaurosis has shown some restoration of photoreceptor function by electroretinography (ERG), as well as structural integration and evidence of local synapse formation within the host outer nuclear layer.[68]

Functional assessment of vision, however, remains difficult in SC studies. Both studies that demonstrated "functional improvement"[12,55] compared treated eyes with untreated eyes at similar time points rather than the same eye before and after treatment. This approach does not exclude the possibility that the transplanted cells had neuroprotective rather than regenerative effects.

The generation of photoreceptor precursors from iPS,[50] which have biological behaviour indistinguishable from that of ES,[53] has become the focus of much recent work.

This has led to the development of differentiation protocols to produce cells expressing photoreceptor-specific markers,[69–72] with the potential benefit of developing specific donor cells for transplant. However, although iPS may circumvent the ethical controversies associated with ES, there are still concerns regarding their safety, including the possibility of carcinogenesis.[73]

Adult-derived retinal SC offer the exciting potential of autologous therapy. Studies in rodent models of retinal degeneration have shown that Müller glia are able to differentiate into photoreceptors.[74] Given that this cell type may be critical for regeneration in the chick[75] and zebrafish[76] retina, the discovery of a similar population of cells in the adult human eye that exhibit SC characteristics[43,44,77] may provide an opportunity to develop transplant strategies to replace photoreceptors in the future.

CAN SC REPLACE RPE?

The RPE supports many of the metabolic functions of the retina, and its dysfunction is associated with degeneration of photoreceptors in AMD and in certain types of RP. This observation has led to extensive work regarding the feasibility of re-establishing the normal interaction between the RPE and photoreceptors via subretinal RPE transplant.[78] Both animal models and human trials have demonstrated the potential of improving visual function. Full-thickness autologous RPE grafts have achieved some success, but investigators are now turning to SC-derived RPE grafts as a renewable source of transplant material.

RPE cultures derived from hES exhibit the appropriate morphology, markers, and functionality in terms of the ability to phagocytose photoreceptor outer segments in vitro.[79] Similar populations can be obtained from human iPS, which exhibit these features both in vitro[80,81] and in vivo[81,82] in rodent models of retinal degeneration. The translation of such therapies to the clinic is often limited by the rigorous safety data required by regulatory agencies.[83] However, such data are available for hES-derived RPE, and results of preclinical transplantation studies are expected in the near future, given that this is one of the first areas to attract investment from the largest pharmaceutical companies and has therefore attracted much media publicity.[84,85]

CAN SC REPLACE RGC AND GROW A NEW OPTIC NERVE?

Compared with photoreceptor replacement, the successful integration of new RGC is more complex. Experimental SC-mediated increased survival of RGC in glaucoma and ON lesion models has been reported. BM-derived MSC and oligodendrocyte precursor cells promote RGC survival through secretion of neurotrophic factors.[86,87] Ensheathment of axons by OEC may also support damaged RGC. Following transretinal injection into healthy rat eyes, OEC migrate within the retina and along the RGC axon layer into the ON head and appear to ensheath RGCs and axons with their cytoplasm.[23] This approach may provide a small degree of axonal rescue of compromised axons (although not regrowth), which may be sufficient to restore a significant amount of functional vision in the clinical context of end-stage glaucomatous and other ON disease.

Successful RGC replacement, however, requires not only migration and integration of donor cells into the ganglion cell layer and differentiation into RGC-like cells, but also the extension of long axonal processes into the ON, through the lamina cribrosa and through the myelinated part of the ON. Myelin-associated glycoproteins strongly inhibit outgrowth of regenerating RGC axons in axotomy models, but this inhibition can be overcome by a combination of neurotrophins and inactivation of specific signalling pathways.[88,89]

As for potential donor cells, ES and adult-derived SC have been investigated as potential RGC replacements; iPS have not yet been used in this context. ES,[90–92] MSC,[93,94] AHPC,[27,37–41] retinal progenitor cells, [25,26] and Müller SC[95] migrate and integrate into retina depleted of RGC or populated by apoptosing RGC.

Some cells even migrate through the lamina cribrosa and into the ON,[41] or extend long processes into the ON.[27,37] Using cell morphology and expression of characteristic markers on immunohistochemistry as indicators of differentiation, all transplanted cell types are capable of expressing proteins characteristic of neurons or glia. However, functional synapses within the retina have never been demonstrated, although synapse-like structures, synaptic vesicles, and expression of the synaptic protein synaptophysin have been described.[38,37] In addition, most studies have not assessed functional outcomes that would demonstrate preservation or restoration of vision, such as ERG response or visually

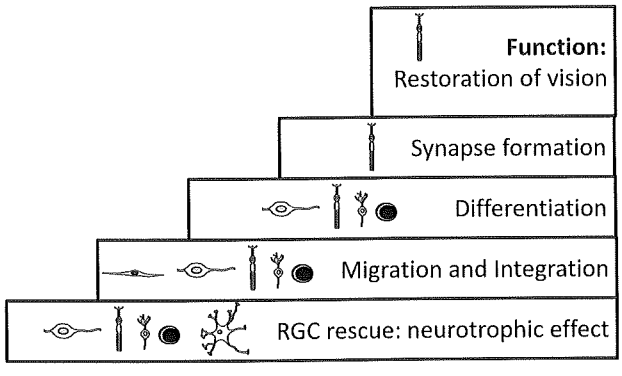

Fig. 4—Steps towards successful photoreceptor and retinal ganglion cell (RGC) replacement. A range of cell types mediate photoreceptor and RGC rescue in animal models. Embryonic stem cells, adult hippocampal precursor cells, retinal progenitor cells, olfactory ensheathing cells, and Müller cells migrate and integrate. Some cell types express markers of differentiation into neuronal and glial cells. However, synapse formation and resumption of function with restoration of vision have only been achieved with postmitotic photoreceptors. (Figure composed using Motifolio Inc. diagrams.)

evoked potentials. The 2 exceptions found that ES and AHPC transplant did not improve visually evoked potentials[91] or ERG response[40] when compared with controls.

What other barriers hamper SC transplantation?

One important finding across many studies of retinal cell replacement is that these strategies only work in damaged retinas or visual pathways.[25–27,37–41,90,91,94–97] It appears that extracellular matrix molecules such as chondroitin sulphate proteoglycans form a barrier that prevents SC integration. These molecules are produced by activated microglial cells and astrocytes and are associated with loss of plasticity within the CNS during postnatal development.[98,99] Pharmacological disruption of extracellular matrix by chondroitinase ABC and matrix metalloproteinase 2, and prevention of microglial activity by immunosuppression or glial toxins such as DL-alpha-aminoadipic acid, or combinations of these, enhance migration, integration, and synapse formation of transplanted cells and will be useful adjunctive strategies to SC transplantation.[95,99,100–103]

Conclusions

This review describes examples of the many different cell therapies that could potentially be used for rescue and regeneration of the retina and the ON, and discusses various factors that need to be addressed before cell transplantation can be developed effectively (Fig. 4). However, translation of these findings into clinical therapies has not been achieved yet, mainly because of the lack of appropriate cell sources and the mixed results obtained with the various experimental models. As our knowledge about SC behaviour increases, including state of differentiation in retinal and ON diseases, SC-based treatment might replace rather than rescue retinal neurons. The majority of studies have addressed the differentiation of stem/progenitor cells into photoreceptors and their application to retinal dystrophies, whereas only a small number of investigations have focused on the differentiation of SC into RGC. However, it is anticipated that as the field advances, this area will grow into one of the most exciting areas of human SC therapy, with the real prospect of being able to restore eyesight in humans who are currently untreatable.

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