Stem-cell therapy in retinal disease

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Purpose of review

Stem-cell research is being investigated for the treatment of retina diseases. Cell replacement strategies have the potential to improve vision in patients who were previously considered to be untreatable. This review summarizes progress within the field and obstacles which must be overcome to make stem-cell therapy a viable treatment for select retinal disease.

Recent findings

Researchers have demonstrated that stem-cell transplants can survive, migrate, differentiate, and integrate within the retina. Stem cells from various developmental stages have been used in these experiments, including embryonic stem cells, neural stem cells, mesenchymal stem cells, retinal stem cells, and adult stem cells from the ciliary margin. Not only can these transplants adopt retina-like morphologies and phenotypes, but they have also shown evidence of synaptic reconnection and visual recovery in both animal and human studies. Still, work must be done to achieve higher yields of functioning retinal neurons and to promote better integration within the host retina.

Summary

Although many obstacles remain, stem-cell-based therapy is a promising treatment to restore vision in patients with retina disease.

Keywords

retina transplant, stem cell

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Introduction

The field of stem-cell research holds great potential for the treatment of retinal disease. In all retinal disorders, the loss of photoreceptors and retinal neurons is regarded as an irreversible cause of vision loss. Any effort to restore vision in these cases would require replacement of the highly specialized network of retinal cells. One strategy has been the development of microelectronic implants, which employs an artificial prosthesis to interface with the biological visual pathway [1]. Although this technology has made progress, reconnecting electronic impulses from these devices to the central nervous system is extremely challenging. Another approach to treating retinal diseases is cell replacement by transplantation, which aims to replace missing retinal cells with new ones that reintegrate into the neuronal network of the visual pathway. This strategy can potentially restore sight in conditions that have been considered untreatable. Stem cells, which have the ability to self-renew and to generate multiple cell types, are a promising donor source for retinal repair and regeneration.

Stem-cell therapy has the potential to treat a wide range of retinal diseases. The neuroretina is a complex structure whose health depends on blood vessels and retinal pigment epithelium (RPE), each of which is affected differently in the spectrum of retinal disease. Therefore, three distinct cell types are conceivable targets for future cell therapy in the retina: the neuroretina (photoreceptors, bipolar cells, ganglion cells, glial cells), RPE, and vascular endothelial cells. Depending on the type of retina disease, different cell replacement strategies need to be developed. For instance, end stage vision loss in patients with age-related macular degeneration (ARMD) is characterized by photoreceptor loss and RPE dysfunction. Promising treatments for exudative ARMD have been developed to inhibit choroidal neovascularization; yet, many of these patients, in addition to those with geographic atrophy, suffer vision loss secondary to photoreceptor cell death. Replacement of lost photoreceptors and RPE by stem-cell therapy offers one therapeutic approach to restore vision for these patients.

A large body of scientific research has been conducted in the field of stem-cell therapy for retinal repair. This review summarizes progress within the field and obstacles that must be overcome to make stem-cell therapy a viable treatment. We discuss efforts to generate donor cells for transplantation and implant them into the diseased retina. As photoreceptor and RPE losses

are common endpoints of many retinal diseases, we focus on stem-cell therapy with regard to these cell types.

Photoreceptor transplantation

The neural retina requires complex protocols for stem-cell transplantation due to its multilayer structure and extensive network of synapses between specialized neurons. Not only will these therapies require an understanding of the molecular cues that promote stem-cell differentiation into functional retinal neurons, but they will also depend upon reconnection into the synaptic network of the visual pathway. Of note, photoreceptor replacement is theoretically more feasible than other retinal neurons because it is a sensory cell connected in only one direction. They generate neuronal activity in response to an external stimulus: light. In diseases that initially spare the inner retinal circuitry, transplanted cells could theoretically replace degenerating photoreceptors, reconnect to the remaining inner retina, and restore vision. The preexisting connections between ganglion cells and the lateral geniculate nucleus would theoretically remain intact. In contrast, ganglion cell regeneration would involve remodeling of afferent impulses within the retina as well as reconnecting to the complex system of downstream targets in the central nervous system.

Donor cell sources

The ideal cell population for donor photoreceptors is amenable to expansion, can undergo directed differentiation into retinal neurons, does not produce an immunologic response, and is able integrate, survive, and restore neural activity in the visual pathway. Identifying the molecular mechanisms that promote these processes is crucial because the diseased retina may have lost the developmental cues that allow photoreceptor repair and integration. In fact, a diseased retina is likely to exhibit proinflammatory and gliotic stimuli that must be neutralized before successful retinal transplantation can occur [2].

Embryonic stem cells

One possible donor source are embryonic stem cells (ESC). They are totipotent cells arising from the eight-cell morula stage that can undergo unlimited self-renewal and differentiate into any adult cell type. Researchers have made progress in understanding the developmental stimuli that derive retinal neurons and photoreceptor progenitors from mouse and human ESCs *in vitro* [3–9]. Yet, evidence is still lacking for producing fully functional cells needed for retinal repair [10]. In addition to technical difficulties, ESCs have limitations that include an ethical debate and inherent risks associated with allogenic transplantation. Nonetheless, they do not appear to stimulate a significant rejection response – an important factor given the morbidity associated with transplant rejection drugs. ESC-based platforms must be further investigated as a potentially unlimited cell source for retinal repair and regeneration.

Neural stem cells

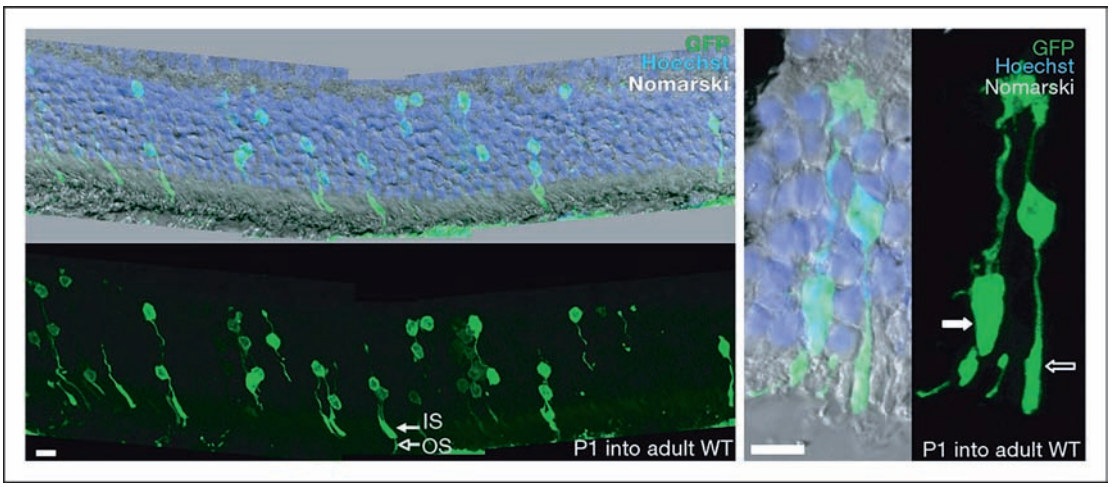
Neural stem cells (NSC) have also been looked at for retinal transplantation. NSCs are self-renewing, multipotent cells that can differentiate into the main cell phenotypes of the nervous system. They have been isolated from the adult mammalian brain tissue, including humans. These stem cells have been transplanted into the adult retina of animal models with evidence of assimilation into host retina [11]. Researchers have also successfully transplanted NSCs by intravitreal injection after they were derived from ESCs in culture [12,13]. Although NSC progeny can adopt retinal-like morphologies and phenotypes, they generally fail to fully differentiate into retinal phenotypes [14–16].

Retinal stem cells

During early development, retinal stem cells (RSC) are a possible donor source that give rise to all retinal cell types. These cells can be isolated, expanded, and differentiated into retinal neurons by culturing them in the presence of growth factors, such as epidermal growth factor and fibroblast growth factor [17–20]. Of interest, when RSCs were transplanted into the subretinal space of the degenerating retina in an animal model, they exhibited preferential expansion as ganglion and glial cells. This study suggested that a diseased retina provides signals that induce RSCs to differentiate into cell types other than photoreceptors. However, other groups have shown that grafted RSCs can demonstrate photoreceptor expression, radial orientation, and some evidence of integration within the host retina [21,22]. In general, RSC can undergo differentiation into retinal neurons; yet, researchers need to make manipulations to enhance retina integration and further characterize whether these cells function as true photoreceptors. Unlike NSCs, they do not grow indefinitely in culture and have to be reexpanded from fetal material. This feature could lead to problems with standardization and reproducibility [23,24].

It is unclear whether the adult retinal environment is able to provide the molecular cues needed to guide a RSC down the pathway to becoming a fully functional photoreceptor. Therefore, investigating retinal progenitor cells from a range of developmental ages can help define the optimal stage at which a donor cell is most likely to integrate into the recipient retina. Multiple groups have shown that postmitotic rod precursors, rather than immature RSCs, displayed a higher probability of integrating into the adult retina [25,26]. MacLaren *et al.* [25] demonstrated that postmitotic rod photoreceptors, rather than

Figure 1 Integration of P1 retinal cells into immature and adult wild-type recipient retinas



GFP-positive neural retinal cell suspensions from P1 transgenic mice migrated and integrated into the outer nuclear layer of GFP-negative wild-type littermate recipients 3 weeks after subretinal transplantation. Most implanted cells were correctly oriented and had morphological features typical of mature photoreceptors. Left. low magnification. Right. Examples of cells with rod (open arrow) and cone (closed arrow) morphologies. IS, inner segment; OS, outer segment; P1, postnatal day 1; GFP, green fluorescent protein; scale bars, 10 μm. Adapted with permission from [25].

immature retinal progenitors, could integrate into the adult retina and restore vision in a rodent model of retinal pigment.

After subretinal transplantation in animal models of retinal degeneration, these cells could migrate into the recipient outer nuclear layer, form synaptic connections, and improve visual function (Fig. 1). Although the degree of photoreceptor incorporation was sufficient to restore a pupillary light reflex, higher levels of integration are essential to improve visual acuity. Using similar post-mitotic rod precursors, one group found that reversible, chemical disruption of the outer limiting membrane enhanced the number of integrated donor photoreceptors [27]. Still, these techniques would be difficult to translate to human stem-cell therapy because an equivalent staged donor cell must come from a second trimester fetus. An alternative approach would be to partially differentiate stem cells *in vitro* before transplantation, allowing them to reach a developmental stage that maximizes their ability to integrate into diseased retina. Researchers have already identified a number of transcription factors that promote differentiation of stem cells into mature retinal neurons *in vivo* [28–34]. This concept of staging cells prior to transplantation will require a better understanding of photoreceptor developmental biology.

Adult stem cells

Stem cells found in adults are attractive candidates for retinal repair by autologous transplantation. If they can be isolated, propagated, and differentiated into photoreceptors that integrate into a recipient retina, complications associated with immune rejection of allogenic tissue and infectious disease could be theoreticall yeliminated.

Recently, researchers discovered a stem-cell population with neurogenic potential within the adult pigmented ciliary margin of the mouse and human eye [35,36]. The role of these ciliary RSCs is still obscure, but they can clonally proliferate *in vitro* and give rise to retinal-specific cell types, including photoreceptors, bipolar neurons, and Müller glia. Furthermore, the progeny of these ciliary RSCs, which have been transplanted via intravitreal injection into neonatal animal eyes, were able to migrate, integrate, and differentiate into photoreceptors and RPE cells [37]. Interestingly, adult mouse RSCs could clonally expand into retinal phenotypes when transplanted into diseased retina, but not in healthy eyes. This finding suggests that injury-induced factors play an important role in promoting the incorporation of RSCs [38]. These exciting studies raise the possibility of isolating autologous RSC for transplantation. Methods for generating viable photoreceptors from adult ciliary RSCs need to be further investigated, as it will be difficult to achieve reproducible protocols for individual patients.

Mesenchymal stem cells (MSCs) are another attractive candidate for retinal regeneration because they can be obtained from the patients' bone marrow in quantities appropriate for clinical application. Although a study found that MSCs differentiated into cells resembling microglia rather than retinal neurons [39], other studies have shown that MSCs differentiate into retinal neurons *in vivo* and *in vitro* [40]. Animal studies have also demonstrated that subretinal transplantation of MSCs delays

retinal degeneration and preserves retinal function [41]. In addition, MSCs can differentiate and incorporate into a host retina with light amplification by stimulated emission of radiation (LASER)-induced tissue injury after intravitreal injection [42].

Fetal retinal cells

Another strategy has been to harvest donor photoreceptors and retinal neurons from the fetal retina when these cells are immature and are primed to form their intrinsic connections. For instance, fetal retina ganglion cells were shown to have the ability to regenerate their axon pathways within the optic nerve and through the optic chiasm after creating a retinal lesion [43]. In another experiment, fetal retinal tissue demonstrated the ability to reconnect to the central nervous system and restore the pupillary light reflex after transplantation into the midbrain [44]. Different approaches to transplantation of fetal retinal cells have been tested. Numerous groups have transplanted whole sheets of fetal neuroretina into the subretinal space of animal models, resulting in evidence of differentiation and long-term survival [45,46]. Other researchers have used dissociated cells and microaggregates from immature retinas. These transplants have developed into viable cells that express photoreceptor molecular markers and are sufficient to mediate simple functions such as a light–dark response [47,48]. Although direct synaptic connections between fetal retinal transplants and host retina can contribute to improvement of visual responses, complete integration of grafted cells continues to be an obstacle for both techniques [49–51]. Of note, some of the visual recovery in these experiments may be attributed to enhanced survival and function of endogenous photoreceptors by means of trophic factors from transplanted tissue [52].

Theoretical disadvantages exist for using full thickness sheets for fetal retina grafts. The presence of inner retinal neurons in the transplant may act as a physical barrier to donor photoreceptors trying to form synaptic connections with host inner retina. Also, transplanted tissue in the subretinal space would separate host photoreceptors and host RPEs, which may render any surviving host photoreceptors nonfunctional. One alternative solution would be to prepare graft tissue by slicing the fetal retina tangentially, which has been described in the literature [53]. This technique would remove unwanted inner retinal components and provide a sheet of oriented, regularly arranged photoreceptors that may be ideal for transplantation.

To date, a few small clinical studies have attempted to transplant fetal retinal tissue in human individuals with advanced ARMD and retinitis pigmentosa. One technique used fetal retinal sheets with attached RPE cells [54], whereas other groups have employed retinal aggregates [55,56]. In all of these studies, the implanted cells were found to be viable and showed no clinical signs of rejection or major complication. However, they had mixed visual outcomes. Although some studies were unable to demonstrate any positive effect on vision, other patients transplanted with fetal retinal sheets showed improved visual acuity over time (up to 6 years) [57••,58]. These results highlight the future potential of fetal retinal grafts. They have demonstrated the capacity to survive, form synapses with host tissue, and improve visual function in some cases.

Method of transplantation

Two methods of stem-cell transplantation, subretinal and intravitreal, have been described and both seem to be effective. The implantation of stem cells into the subretinal space is a more complex, technically demanding procedure, but it places the grafted cells close to their intended location. On the contrary, intravitreal injection is much less invasive and easier to perform, but transplanted cells must migrate from the vitreous cavity to the outer retina. Some studies have shown that NSCs implanted into the subretinal space have better localization and photoreceptor differentiation than intravitreal grafts [13,59]. Other studies have demonstrated that RSCs incorporate better after intravitreal injection compared with subretinal implantation [37]. Both of these techniques have proven efficacious and investigators will continue to experiment with them.

Retinal pigment epithelium transplantation

The RPE is composed of a monolayer of pigmented cells that lies adjacent to photoreceptor outer segments and plays a crucial role in retinal homeostasis. RPE dysfunction occurs in several retinal disorders, such as retinitis pigmentosa and ARMD; therefore, regenerating RPE cells by stem-cell therapy is one promising way to treat these conditions. As RPE cells do not require synaptic reconnection, RPE transplantation may be less complex than that of photoreceptors and retinal neurons. To date, researchers have translocated RPE tissue into the fovea of ARMD patients after choroidal neovascular membrane removal [60]. Patients maintained foveal fixation early, but central visual function was found to be transient, with a decline at 5 to 6 years [61]. Another group transplanted autologous RPE cells harvested from the nasal retina to the macular region in patients with ARMD. This technique led to clinical benefit in some patients, but there was difficulty in collecting a sufficient number of autologous RPE cells [62]. Autologous RPE transplants also have the disadvantage of carrying the same genetic predisposition that led to the original disease state.

Allograft RPE transplants have also been attempted, in which sheets of adult human RPE were successfully

transplanted into the subretinal space of ARMD patients at the time of subfoveal membranectomy. Systemic immunosuppression was used and no rejection was observed. Unfortunately, there was no postoperative improvement in visual acuity at 1 year [63•,64]. One drawback for allogenic RPE grafts is that primary RPE cells cannot be obtained in large enough quantities for wide-scale clinical use. Furthermore, it seems impractical to systematically assess the functional parameters of graft efficacy on every fetal or adult donor source [65]. These issues highlight the importance of exploring stem-cell therapy to create RPE cell lines for transplantation.

One study [66] reported the isolation and characterization of a reproducible source of RPE cells from human ESCs. RPE cells generated using stem-cell technology have been transplanted in a rodent model of retinal disease, in which photoreceptor loss is caused by a defect in RPE [65]. A suspension of stem-cell-derived RPE cells was injected subretinally and shown to localize to the subretinal space without encroachment into the retina. This technique of RPE transplantation demonstrated long-term photoreceptor survival and preservation of visual function as measured by electroretinogram. Ultimately, researchers have proven that RPE can be derived from ESCs under well defined conditions and can be successfully transplanted into animal models of retinal disease.

Obstacles and future prospects

Many unanswered questions surrounding stem-cell transplantation must be addressed. What is the best type of stem cell for retinal transplantation? What is the best method of delivery? What stage of stem-cell differentiation is ideal for integration within the host retina? What extracellular conditions maintain the greatest graft survival? What is the optimal morphologic structure of the stem-cell transplants: entire, intact sheets of retina versus cell suspensions?

Another area of concern is immune rejection for allografts. RPE transplants in rats initiated a mild, chronic rejection [67] despite the immunopriviledged status of the subretinal space [68]. Although rejection was less severe than typical mismatched tissue allografts, this finding raised the question of the need for immunosuppressive therapy. Further developments, such as the creation of reduced complexity human leukocyte antigen (HLA) stem cells and somatic cell nuclear transfer (SCNT), may help overcome the problem of immune rejection. SCNT, also known as therapeutic cloning, is a technique to generate stem cells that are genetically matched to the recipient patient. The procedure involves removing the nucleus from an unfertilized egg cell, replacing it with nuclear material of a somatic cell from the recipient patient, and stimulating it to divide into a stem cell [69].

Transplantation of stem cells that undergo continued proliferation carries the theoretical risk of teratoma formation. Tumor growth after transplantation of a neurally selected ESC in a rodent model has been reported [70]. Although this finding appears to be isolated, cell-sorting techniques may be necessary to isolate generated retinal cells from potential tumor cells.

Ongoing disease in the host environment may prevent stem-cell transplants from successfully differentiating and assimilating into the recipient retina. In many retinal diseases, degeneration of photoreceptors triggers extensive remodeling, changes in neuronal migration, rewiring of synaptic connections, and fibrotic and pigmentary alterations [71]. The extracellular milieu in a diseased retina may need to be modified before it can be fully amenable to cell-replacement therapy.

Conclusion

There have been exciting advances in the field of stem-cell therapy and retinal disease. Although significant obstacles still exist, the literature clearly demonstrates that stem-cell transplants can adopt retina-like morphologies and phenotypes, as well as show evidence of synaptic reconnection and visual recovery in animal and human studies. Researchers will continue working to achieve higher yields of functioning retinal neurons and to promote better integration within the host retina. In general, the medical and scientific community is optimistic that stem-cell-based therapy to restore vision is becoming closer to reality.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 226).

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