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Review Article

Retinal stem cells and potential cell transplantation treatments

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Abstract

The retina, histologically composed of ten delicate layers, is responsible for light perception and relaying electrochemical signals to the secondary neurons and visual cortex. Retinal disease is one of the leading clinical causes of severe vision loss, including age-related macular degeneration, Stargardt's disease, and retinitis pigmentosa. As a result of the discovery of various somatic stem cells, advances in exploring the identities of embryonic stem cells, and the development of induced pluripotent stem cells, cell transplantation treatment for retinal diseases is currently attracting much attention. The sources of stem cells for retinal regeneration include endogenous retinal stem cells (e.g., neuronal stem cells, Müller cells, and retinal stem cells from the ciliary marginal zone) and exogenous stem cells (e.g., bone mesenchymal stem cells, adipose-derived stem cells, embryonic stem cells, and induced pluripotent stem cells). The success of cell transplantation treatment depends mainly on the cell source, the timing of cell harvesting, the protocol of cell induction/transplantation, and the microenvironment of the recipient's retina. This review summarizes the different sources of stem cells for regeneration treatment in retinal diseases and surveys the more recent achievements in animal studies and clinical trials. Future directions and challenges in stem cell transplantation are also discussed.

Keywords: ciliary marginal zones; embryonic stem cells; induced pluripotent stem cells; retinal diseases; stem cells

1. Introduction

The eyes are a specialized sense organ extending from the central nervous system that receives light stimulation at retinal photoreceptor cells and relays electrochemical signals to the visual cortex. The pathway of light from the outermost to the innermost receptors of the eyes passes through several ocular tissues, including transparent corneal tissues, aqueous humor, the pupil, the lens, the vitreous layer, and ten layers of retinal tissue. After the photoreceptor cells in the outer retina perceive light, chemical energy is converted into electrical impulses and is then transmitted to the brain through the axons of ganglion cells occurring in the inner retina as bundles of nerve fibers.

The retina is comprised of ten histological layers in the following sequence: inner limiting membrane; nerve fiber layer; ganglion cell layer; inner plexiform layer; inner nuclear layer; outer plexiform layer; outer nuclear layer; external limiting membrane; photoreceptor layer; and retinal pigment

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epithelium (RPE). The inner nuclear layer contains the nuclei of the bipolar cells, horizontal cells, and amacrine cells as well as Müller cells; the outer nuclear layer contains the cell bodies of rods and cones. The inner plexiform layer comprises the synapse between the ganglion cell layer and the inner nuclear layer; the outer plexiform layer contains the synapse between the inner and the outer nuclear layers. At the cellular level the retina consists of six classes of distinct neurons (cone and rod photoreceptors, horizontal, bipolar, amacrine and ganglion cells, Müller glial cells, and astrocytes) responsible for light perception, signal integration, and tissue support/repair, respectively.

Retinal regeneration after neuronal injury takes place in several species of vertebrate, including fish, amphibians, birds, and mammals. It has been assumed that retinal stem cells (RSCs) or precursor cells are situated in the ciliary marginal zone (CMZ), from which the rod precursors or Müller glial cells may be recruited for retinal regeneration. As an extreme example, retinal regeneration in teleost fish can occur even after surgical removal of a portion of the central retina. However, intrinsic retinal regeneration, occurring in fish and chick embryos by the formation of new retinal neurons from progenitor or stem cells in the CMZ, does not occur in mammals, including humans.[1,2] By contrast, mammalian Müller glia proliferate and give rise to neuronal cells in response to retinal damage, but the regenerative capacity is more limited than that in fish and birds.[3]

In humans, neural stem cells and progenitor cells that maintain their extended passage *in vitro* have been identified in the brain, spinal cord, and fetal retina.[4–8] Human retinal progenitor cells isolated from the fetal retina are capable of dividing for multiple generations and differentiating to several retinal cell phenotypes, indicating that these cells have the potential to be used therapeutically to replace damaged retinal neurons. However, the availability of fetal retinas and associated ethical concerns may limit the extensive use of embryonic stem cells (ESCs) in treating retinal diseases.

RSCs have been assumed to be in the pigmented or non-pigmented epithelial layer of the ciliary margin at the peripheral edge of the retina. As most of the differentiated lineages are photoreceptor cells, transplantation of RSCs has shown potential as a tool for cell treatment in degenerative retinal diseases.[1] In this paper we review the different sources of retinal and somatic stem cells and the current trends in applying these versatile stem cells to cell transplantation.

2. Endogenous retinal stem cells

A population of retinal stem cells in the ciliary body of mouse and human eyes has been identified as possessing the ability to proliferate *in vitro* and produce all of the retinal cell types.[7,9,10] However, other evidence has shown that these cultured retinal stem cells from the ciliary body are actually RPE cells and lack the ability to generate the differentiated retinal lineages, particularly photoreceptor cells.[11,12] However, RSCs harvested from porcine and murine retinas have been shown to restore visual functions after allogeneic retinal transplantation,[13] thus the real properties of these cells still need to be clarified.

2.1. Neural stem cells

The adult mammalian central nervous system contains endogenous neural stem cells (NSCs) capable of proliferation and further differentiation. The partially differentiated progenitor populations isolated from the eye or the brain may be a potential source for retinal cell transplantation. Our previous study compared the proliferation and differentiation ability of RSCs and NSCs, showing that the latter had a higher proliferating potential at the 10th week, in contrast with RSCs, which showed interrupted proliferation at the 8th week.[14] We also found that the combination of transforming growth factor beta type III with retinoic acid played an important role in the induction of NSCs to differentiate into opsin-positive cells. The cells integrated into the outer retinal layer will usually generate mature photoreceptor cells based on the cellular morphology, expression of photoreceptor markers, and functional recovery.

2.2. Müller cells

Müller cells are the major type of glial cells in the retina and are responsible for the homeostatic and metabolic support of retinal neurons. These cells are located across the entire retinal layer, from the vitreous surface to the subretinal space. Their function is thought to be the mediation of transcellular ion, water, and bicarbonate transport.[15] However, the exact functions of Müller cells remain unknown. Müller cells functioning as retinal stem cells in mammalian eyes have been reported previously.[15] Subsets of Müller cells from mature retinas have been found with the properties of neural progenitor or stem cells.[16]

Müller cells in zebra fish present the multipotent progenitor marker, Pax6,[17,18] and can be induced to dedifferentiate, proliferate, and generate neural stem cells that migrate to damaged retinas and further differentiate into neurons in response to injury.[19] Müller cells from zebra fish also play an essential role in retinal growth, even in adulthood.[18] In a study of chicks, it was found that injury to the retina induced Müller cells to re-enter the cell cycle and produce neurofilaments and transcription factors which could only be found with an embryonic status.[20] Müller cells from the retina of chicks have the potential to regenerate all types of retinal neuron in response to injury, but this potential decreases with the age of the animal.[21] Karl et al[22] found *in vivo* evidence that Müller cells have the potential to proliferate and differentiate into new neurons in the damaged retina of mice.

As a result of their potential to generate neuron stem cells, Müller cells have been regarded as a potential cell-based strategy for retinal regeneration. Some studies have shown that transdifferentiated Müller cells could be cultured from NSC markers expressing Müller cells extracted from mature human retina, the epiretinal membrane of proliferative vitreoretinopathy patients, and human retinal cell lines.[23–26]

Transplanting these cells via either subretinal or intravitreal injection can lead Müller cells to migrate into the retinal parenchyma and express neuronal cell markers.[23]

2.3. Pigmented ciliary marginal zone

The two cuboidal epithelia of the ciliary body derived from the neural tube consist of the outer pigmented layer, continuous with the RPE, and the inner nonpigmented layer, or nonpigmented epithelium, continuous with the neural retina.[27] The ciliary marginal zone (CMZ) is a ring of cells at the periphery of the maturing and matured neural retina that contains RSCs and progenitor cells. Retinal neurons are added to the periphery of the retina by differentiation of these nonpigmented CMZ RSCs and associated progenitor cells. The ciliary body is anteriorly adjacent to the CMZ and parts of it, as well as the retina and CMZ, are derived from the optic cup.

Tropepe et al[7] detected the presence of retinal stem cells in the pigmented ciliary margin of the eyes of adult mice. These cells show the multipotency of proliferation and self-renewal, and can differentiate into bipolar neurons, Müller glia, and photoreceptors. Liu et al[14] showed that RSCs were also localized in the pigmented ciliary margin of adult rats. These RSCs have the potential for self-proliferation and multipotent differentiation. Similar to RSCs found in the pigmented ciliary margin of adult rodent eyes, pigmented cells at the retinal margin of postnatal chicks were also noted to have the same stem cell potential as those from embryonic retina.[28]

3. Potential of retinal differentiation from other somatic stem cells

3.1. Bone marrow mesenchymal stem cells

Bone marrow mesenchymal stem cells (BMSCs) belong to one of the established stem cell lines and the protocols for cell purification, amplification, and transplantation are well defined. The mechanism of repair in damaged tissue by BMSCs was found via fusion with the host cells instead of incorporation and transdifferentiation.[29,30]

Although BMSCs have the potential to rescue damaged retinas, only a small portion of transplanted cells may integrate into the host retina, while the rest remain in the vitreous cavity without engrafting because of glia reactivity.[31] To promote the stem cell mediated regeneration of RPE, there are three approaches to deliver BMSCs into targeted retina.[32] The first is the endogenous delivery of BMSCs combined with pharmacologically enhanced growth factor mediated mobilization, leading to the migration of cells into the subretinal space.[33] Second, the transplantation can be accomplished by an exogenous approach. The third approach is subretinal injection; BMSCs injected into the subretinal space may be integrated into the RPE layer and express differentiation markers.

BMSCs can differentiate into neural precursor cells (nestin-positive) and mature neurons (MAP-2 and Tuj1-positive) following treatment with neural selection and induction media. Furthermore, BMSC applications are not limited to repairing damaged RPE cells; our recent results showed that BMSCs can have multipotential differentiation and differentiate into photoreceptors and retinal cells after co-culture with RPE cells. This platform provides us an *ex vivo* expansion model of culturing photoreceptors for the treatment of retinal degeneration diseases.[34]

3.2. Adipose-derived stem cells

Adipose tissue has been viewed as another source of stem cells with several advantages. First, adipose-derived stem cells (ASCs) are easily acquired in huge volumes via a relatively pain-free, smooth approach. Second, ASCs can be repeatedly harvested under local anesthesia.[35] Third, ASCs show better proliferation potential, are more easily separated by enzymes, and have a longer life span than BMSCs.[36] Currently, ASCs are widely used for cosmetic purposes, such as breast reconstruction, with relatively few safety concerns compared with other sources of stem cells.

Applications of ASCs in ophthalmology are still in progress. According to some studies, ASCs may be induced to differentiate into corneal epithelial-like phenotype, keratocyte, and even retinal progenitor cells.[37–39] ASCs have been found to indirectly protect light-induced retinal injury through their conditioned medium without cell transplantation, suggesting that ASCs help to protect the retina from damage via secreted proteins.[40]

4. Embryonic stem cells and induced pluripotent stem cells

ESCs derived from the inner cell mass during the blastocyst stage of embryos are capable of self-renewal indefinitely and remain pluripotent to generate all the specialized cell types, which have been shown to be valuable as donor cells for retinal regeneration.[41–43] However, ESC-based regeneration treatment is restricted by the ethical issues of using human embryos and the possibility of immune rejection after ESC transplantation.

Similar to the properties of ESCs, it has been found that induced pluripotent stem cells (iPSCs) can be induced from somatic cells with the introduction of four transcription factors, namely *Oct-3/4*, *Sox2*, *c-Myc*, and *Klf4*.[44] iPSCs present many characteristics equivalent to those of ESCs, such as cell morphology, proliferative abilities, surface antigens, gene expressions, the epigenetic status of pluripotent cell-specific genes, telomerase activity, and teratoma formation.[45,46] iPSCs can differentiate into cell types of three germ layers that have enormous therapeutic potential for regenerative medicine.[47]

Concerning the possibility of tumorigenicity, the introduction of four transcription factors, including *c-Myc,* is thought to reactivate the c-*Myc* virus by overstimulating cell growth and metabolism and/or by causing genomic instability.[48] Dysregulated *c-Myc* expression is associated with several human cancers and is often linked with a poor prognosis.[49] An earlier study successfully generated iPSCs from mouse and

human fibroblasts by using only three transcription factors, *Oct-4, SOX2*, and *Klf4,*without *c-Myc*. Mice treated with the iPSCs without *c-Myc* (non-*c-Myc* iPSCs) did not develop tumors during the study period.[50] Our recent study reported that subretinal transplantation of non-*c-Myc* iPSCs effectively reduced retinal ischemia and reperfusion injury in a rat model via the regulation of oxidative parameters and the paracrinal secretion of trophic factors.[51]

5. Transplantation of stem cells

Treatment based on stem cells mainly depends on the pathogenesis of the disease and the cell resources available for transplantation. In previous reports, a retina with photoreceptor degeneration could be repaired by the transplantation of photoreceptor precursors or ESC-derived progenitors, which were able to form synaptic connections with the host retina and improve visual function.[52,53] In addition, the transplantation of ESC-derived RPE has been reported to improve visual function in RPE degeneration diseases, such as prevailing age-related macular degeneration (AMD).[54,55] John et al[2] summarized the choices of cell in regeneration treatment for AMD. In Grade I AMD with only RPE degeneration, transplantation of RPE alone is preferred. In Grade II AMD with RPE and photoreceptor damage, BMSCs, with their ability to differentiate into photoreceptors, have been suggested. However, BMSCs are avoided in the treatment of wet AMD because of their angiogenic potential. Instead, some workers have successfully transplanted photoreceptor precursors into rod-deficient mice or mice with inherited photoreceptor degeneration.[56,57] In Grade III AMD involving the RPE, photoreceptors, and retinal neurons, BMSCs, ESCs, and iPSCs are all possible choices for transplantation based on their ability to be induced and differentiated into retinal neurons. There are currently two clinical trials on the subretinal transplantation of ESC-derived RPE to confirm the safety and tolerability of this technique in Stargardt's disease and patients with dry AMD. However, ethical concerns and immune rejection problems remain major issues with regard to these cell sources. Some studies have shown that iPSCs could promisingly restore retinal structure and function in mice with retinal degeneration.[58]

The application of human pluripotent stem cells as a donor cell source for transplantation treatment requires well-defined and controlled differentiation conditions. Unlike somatic stem cells with limited propagates, in theory ESCs and iPSCs have the ability self-renew indefinitely. If photoreceptors and/or RPE can be differentiated from human ESCs and iPSCs under well-defined conditions without safety concerns, they will have great potential for the repair of retinal degeneration.

The correct cell type and timing for harvesting donor cells is critical for successful transplantation. For instance, one study has shown that the integration of donor rod photoreceptors into the host retina requires the rod photoreceptors to be taken from the developing retina at a time coinciding with the peak of rod genesis.[53] These studies suggested that the ontogenic-staged transplanted photoreceptors could integrate, differentiate into rod photoreceptors, form synaptic connections, and improve visual function, further demonstrating the importance of cell type-specific and stage-specific purification of the differentiated ES and/or iPS cells. The selection of specific types of ESC-derived progenitors for transplantation into host mice relies on the identification of surface antigens coding rod photoreceptors on postnatal Days 3–6 and the purification of ES and iPS cell-derived rod photoreceptors corresponding to the postnatal stage of Days 3–6.[59] The host environment is also crucial for successful photoreceptor transplantation.[60]

Retinal degeneration is well known, together with the corresponding microglial activation and glial scar formation that impedes the integration and survival of transplanted cells. The robust integration of transplanted retinal cells into the retinas of host mice without the assistance of both vimentin and glial fibrillary acidic protein has been reported.[61] Moreover, matrix metalloproteases and chondroitinases that degrade the extracellular matrix in the diseased retina assist in the integration of transplanted photoreceptors.[62,63] The disruption of the outer limiting membrane also increases photoreceptor integration following transplantation.[64] These studies show that the glial barrier in the host retina prevents the integration of donor photoreceptors. Therefore, in addition to immunosuppression, the host retinal environment must also be modulated for successful transplantation.[65]

Although evidence has shown that stem cells are capable of differentiation into specialized tissues under certain conditions, the question of how to apply these techniques into actual regenerative medicine is still open. Such studies are important to establish: (1) the principles of cell replacement treatment; (2) that the recipient retina is indeed receptive to cells from an external source; and (3) that the retinal microenvironment can support and maintain foreign cells under certain conditions. Immunological and tumorigenic concerns limit the therapeutic use of ESCs. Although BMSCs are free from ethical concerns, they are usually only available in very limited amounts and are difficult to harvest. ASCs provide future potential for regeneration treatment that has so far received scant study. iPSCs are an alternative route to maintain cells that are undifferentiated for prolonged periods of time, to expand cell numbers, to promote differentiation along a desired lineage, and to avoid tumorigenicity in future differentiation directions. However, more efforts need to be made on disease-specific and patient-tailored stem cell transplantation for retinal regeneration to translate the technology of iPSCs into a clinical setting.

In conclusion, research in retinal stem cells is an excellent approach to understanding how concepts and methods of regeneration medicine can be safely and efficiently used in treating retinal diseases. Although the vertebrate retina has, over recent decades, been well characterized and proposed as a good model for studying neurogenesis, clarification of the detailed mechanisms involved in retinal regeneration and efficient methods to control the differentiation of pluripotent cells still requires further investigation. In this review, we have discussed several potential cell sources for regeneration treatment that have arisen from various mammalian reprogramming studies in clinical settings (Fig. 1). Success in

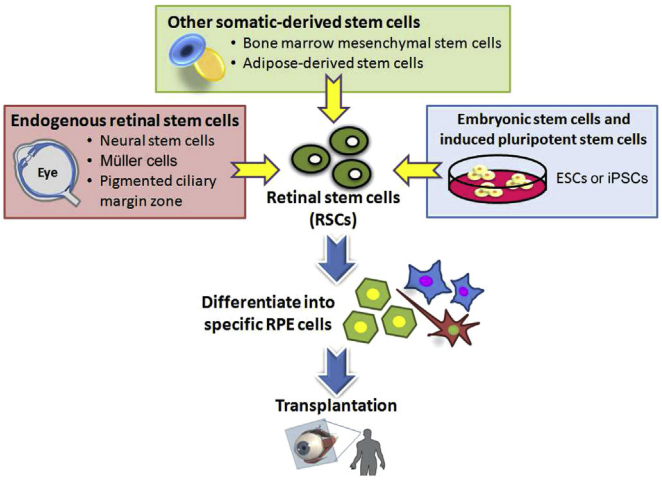

Fig. 1. Cell sources for stem cell transplantation treatment of retinal diseases.

generating the desired cell types by direct reprogramming may benefit from the selection of proper cell sources, the correct timing of the applications, and the nourishing microenvironment on which the transplanted cells depend. All of these efforts offer feasible and promising results for patients to take advantage of retinal transplantation in the future.

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