Stem Cell Based Strategies for the Treatment of Degenerative Retinal Diseases

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Differentiations into the retinal precursors from stem cells are being actively investigated. However, at present, there are no effective therapies for treatment of the retinal cell loss and blindness, such as macular degenerations and hereditary retinal diseases. The retina is part of the central nervous system and contains neurons that convert light into neural signals, which are then transmitted to the brain. The retina plays the most important role in the visual pathway. As such, retinal diseases are important causes of blindness in developed countries.1 Fundamental to visual recovery is successful replacement of damaged retinal cells with functioning retinal cells.

Although significant progress has been made with electronic implants in recent years, an interface between the electronic hardware and biological tissues is still required at some point along the visual pathway. Conversely, synaptic reconnection of transplanted embryonic neurons is an innate biological property that requires...
little manipulation. Although electronic devices are extremely effective in converting visual images into a series of electrical impulses, the issue of reconnection to restore sight still exists. Therefore, a cell-based approach is far more feasible as a potential clinical treatment.

Stem cells can be induced to differentiate into two kinds of retinal cells: photoreceptors and retinal pigment epithelia. While photoreceptors are sensory neural cells that convert light into neuro-electrical signals, retinal pigment epithelia play critical roles in maintaining the neuro-retina.

**Induction of retinal cells from fetal tissues**

Compared to adult neural cells, fetal neural cells survive better after transplantation. Good integration with retinal tissues can be expected when the photoreceptors are harvested, as fetal retina forms connections with neural tissues. Fetal retinal 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. Similarly, developing neural cells have the ability to significantly regenerate the nervous system after an injury because of their high plasticity. Therefore, immature photoreceptors are expected to reconnect better than mature photoreceptors when transplanted to the retina. In a recent study using a retinal degenerative animal model, transplantation of fetal retinal tissues was reported to form neural connections in the retina, which led to partial recovery of visual function.

**Induction of retinal cells from embryonic stem cells**

Ethical considerations aside, fetal tissues are in very limited supply and do not represent a consistent source for retinal cell transplantation. Embryonic stem cells, which undergo unlimited self-renewal, can be an alternative source for photoreceptors. Compared to adult stem cells, they have some advantages as optimal donor cells, including high plasticity and migrating abilities. While photoreceptor differentiation from human embryonic stem cells (hESC) is fundamental to the treatment for retinal degenerative diseases, the research is still in its infancy, and successful differentiation into functional photoreceptor has not yet been reported.

It has been reported continuously that differentiation of embryonic stem cell into primitive photoreceptors and retinal pigment epithelium (RPE). In 2006, Banin et al. reported that hESC-derived neural precursors migrated far away and expressed photoreceptor markers, such as rhodopsin and blue cone opsin after transplantation into a mouse retina. Lamba and his colleagues also differentiated the hESC into the retinal neurons using a combination of noggin, dkk1, and IGF-1 that promoted neural differentiation. Although differentiated cells exhibit a gene expression profile similar to progenitors derived from human fetal retina, differentiation into photoreceptor in vitro is not efficient.

In 2004, Klimanskaya et al. first reported differentiation of retinal pigment epithelium from hESC. Thereafter, other investigators modified culture conditions to derive RPE. They also transplanted differentiated cells at different differentiating step to the animal models in order to confirm cell survival and RPE markers expression. Figure 1 shows hECS and the differentiated RPE cells which express RPE markers in our laboratory.

Co-culture with retinal tissues has been widely used to induce photoreceptors and RPE from ESCs, because co-culture provides favorable environment for stem cells to differentiate into retinal neurons. Recently, Osakada et al. reported induction of RPE and photoreceptor from ESCs in the absence of co-culture with retinal tissues in 2008. However, this method requires long differentiation time and is inefficient. Moreover, the
researchers did not perform functional analysis on the animal models.

Although ESCs have many advantages, such as totipotency and abundance as a source of retinal cells, ethical issues and teratoma risks remain its major concerns.

**Induction of retinal cells from adult stem cells**

Adult stem cells are attractive candidates for retinal repair. They have characteristics of stem cells, such as self-renewal and differentiation, yet exhibit limited self-renewal compared to ESCs. Neural stem cell can be isolated from the brain of adult vertebrates. In 1992, neural stem cell was first isolated from mouse brain striatal tissue. In addition, stem cells that can differentiate into neural cells were identified in the eye of a mouse and a human. These results created a sensation, suggesting that cell therapy is possible via autologous transplantation in retinal diseases. Table 1 shows stem cells related to eye tissues.

While neural stem cells transplanted into adult retina have shown some evidence of being able to integrate into the host retina, they have thus far failed to differentiate into retinal phenotypes. Conversely, retinal-derived stem cells differentiate into retinal phenotypes, but appear to lack the ability to migrate and integrate into the host adult neural retina. It is assumed that adult retina constituted an environment that inhibited retinal progenitor cell integration and differentiation, possibly due to a lack of extrinsic cues that are present during development. As such, cells able to differentiate into photoreceptors in an adult retina are at a specific stage of development: that is when they are already committed to a photoreceptor fate.

**Intrinsic and Extrinsic Factors related to retinal cell differentiation**

Various cell types can be a source for replacing retinal neurons. To facilitate and confirm their potential to be...
developed into retinal progenitors, deeper understandings of the factors that involve in the development of retinal cells is crucial.

Neuronal specification is guided by complex interactions between intrinsic programs and extrinsic regulatory factors, and requires precise coordination between exiting the cell cycle and differentiation. The Pax-6 gene encodes a transcription factor that controls ocular development. Chx10 is one of the earliest markers in the presumptive neural retina of the invaginating optic vesicle. Mutations in both human and mouse Chx10 genes lead to microphthalmia. Chx10 activates the transcription factor gene Crx, which in turn activates NeuroD and neural retina leucine zipper (Nrl). Crx and NeuroD are expressed in the photoreceptors of the developing and mature retina, and are essential for correct differentiation and maturation. Nrl is exclusively expressed in rod photoreceptors, and is indispensable for their development and maintenance. In vitro studies indicated that Nr2e3 acted synergistically with Nrl and repressed the activation of cone genes by Crx. At present, it remains to be elucidated how precise the timing of these transcription factors needs to be, and indeed whether certain steps can be bypassed. It remains unclear whether true differentiation of photoreceptors can occur in vitro, because many of the required developmental cues arise outside the cell in the complex milieu of the primitive eye cup.

Generation of cells exhibiting features of retinal precursors has been achieved through a diverse range of procedures performed on mouse and human embryonic stem cells. In addition, co-incubation of these retinal progenitor-like cells with retinal explants was shown to trigger their differentiation into cells expressing photoreceptor specific markers.

Ikeda et al. and colleagues induced differentiation of mouse embryonic stem cells into photoreceptor progenitors using lefty-A, Dkk1 and Activin A. They found that 25~30% of cells expressed Pax6 and Rx. The cells, upon differentiation by co-culture with adult retinas, formed rhodopsin and recoverin expressing photoreceptors. Human ES cells can also be directed to a retinal cell fate using a combination of a bone morphogenetic protein (BMP) inhibitor, a wingless-type MMTV integration site family (Wnt) inhibitor and IGF-1. The cells differentiated under this protocol also expressed early markers of photoreceptor differentiation, such as Crx, Nrl, and recoverin.
Osakada et al.\textsuperscript{15} described for the first time an efficient, stepwise, procedure for producing large quantities of photoreceptors from mouse ES cells under defined conditions. They first generated Rx-positive retinal progenitors by sequentially exposing ES cell aggregates to Wnt and Nodal antagonists, activin and serum. The authors then established that inhibition of Notch signaling efficiently converts Rx-positive cells into post-mitotic Crx-expressing photoreceptor progenitors. Switching them into a retinal culture medium then promotes significant generation of differentiated progeny, expressing cone-specific pigment proteins with a minority expressing the rod opsin marker. Selective differentiation into rhodopsin-positive cells could be enhanced upon treatment with a cocktail of fibroblast growth factors (FGFs), Sonic Hedgehog, taurine and retinoic acid.

The role of each extrinsic factor on differentiation to retinal progenitors ought to be further investigated, in order to develop differentiation protocols with higher efficiency.

**Method of transplantation**

Safe and effective modes of transplantation are essential in instituting successful stem cell therapy for retinal diseases. Plausible routes for transplantation of retinal cell are 1) trans-scleral 2) intravitreal and 3) sub-retinal transplantation (Figure 2).

Implantation of stem cells with a trans-scleral approach is usually conducted on mice and rats with very small eyeballs, as Wang et al.\textsuperscript{31} described. First, the operator punctures the sclera with a fine needle or blade. Second, a glass pipette or a fine needle will be used to approach the sub-retinal space to implant stem cells. Although this method places the grafted cells close to their intended location, it is a technically demanding procedure with potentially serious complications, such as intraocular hemorrhage and retinal detachment.\textsuperscript{17}

Intravitreal injection is much less invasive and easier to perform. However, the transplanted cells must migrate from the vitreous into the outer retina by themselves. The injected cells can integrate only into early postnatal retina or degenerated adult retina.\textsuperscript{16,32} Intravitreal implantation can be used for conditions, in which the trans-scleral approach is difficult, for example in neonatal mice.\textsuperscript{33}

Transvitreal-sub-retinal implantation can be used in mid- or large-sized animals, or indeed in clinical trials. In this procedure, vitrectomy should be performed prior to the sub-retinal approach. A smooth, flexible needle is
introduced through retinotomy into the sub-retinal space to implant cells. The procedure requires a skilled retinal surgeon.34

A previous study demonstrated that stem cells incorporated better following intravitreal injection, compared to sub-retinal implantation.35 In contrast, some studies showed that stem cells implanted into the sub-retinal space had better localization and photoreceptor differentiation than intravitreal grafts.10,19 Both of these techniques have proven efficacies and investigators can select an appropriate method for their experiments.

Summary

Stem cell based therapy for vision-threatening retinal diseases is under very active investigations at present. It should be a fundamental treatment option to replace damaged retinal cells in the near future. To utilize stem cells in patients with degenerative retinal diseases, more thorough understanding of retinal cell development and the key regulators involved is crucial. Moreover, a more efficient and standardized methodology for stem cell differentiation, and evaluation of functional and anatomical outcomes post-transplantation, should be determined. Optimized animal models should be further investigated.

REFERENCES


= Abstract =

Stem-cell therapy has the potential to improve vision in patients with untreatable retinal disease. Various types of cell source including fetal, embryonic and adult stem cells, intrinsic and extrinsic factors for differentiation into retinal progenitors and transplantation mode were discussed in this review. Experimental approaches have successfully induced photoreceptor precursor cells and retinal pigment epithelium. Stem-cell-based therapy is a promising treatment to restore vision in patients with retinal disease, in spite of the challenges.

Key Words: Stem cells, Stem cell transplantation, Retinal degeneration, Retinal pigment epithelium, Photoreceptor cells