# Chapter 72
# Regenerative Medicine: Solution in Sight

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**Abstract** The retina, like other central nervous system tissues, has poor regenerative properties in humans. Therefore, diseases that cause retinal cell loss, such as Age-related macular degeneration (AMD), retinitis pigmentosa (RP), Leber congenital amaurosis, Usher syndrome, glaucoma, and diabetic retinopathy, typically result in permanent visual impairment. Stem cell technologies have revolutionized our ability to produce neural cells in abundant supply. Much stem cell research effort is focused on producing the required cell types for cell replacement, or to generate disease-in-a-dish models to elucidate novel disease mechanisms for therapeutic development. Here we review the recent advances in stem cell studies relevant to producing RPE and retinal cells, and highlight future directions.

**Keywords** Stem cells · Retina · RPE · hESC · iPSC · Progenitor · Direct cellular reprogramming · Disease modeling

## 72.1 Induction of RPE and Neural Retinal lineages from Embryonic Stem Cells

Since the derivation of embryonic stem cells (ESCs), first in mouse and later in human (Evans and Kaufman 1981; Martin 1981; Thomson et al. 1998), several protocols have been developed to direct ESC differentiation towards RPE and neural retinal progeny. A combination of environmental factors known to stimulate retinal

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development in animal models, including Lefty-A, Dkk1 and Activin A, were used to induce retinal progenitor cells from mouse ESCs, which resulted in 25–30 % $Rx^+$/$Pax6^+$ retina progenitor cells (Ikeda et al. 2005). Pioneering studies using human embryonic stem cells (hESCs) demonstrated that treatment with a combination of BMP inhibitor, Wnt inhibitor and IGF-1 efficiently generated (~ 80 %) human neural retinal progenitor cells (Lamba et al. 2006). A combination of Wnt and BMP/Nodal antagonists was also found effective in neural retina induction from hESCs (Osakada et al. 2008). Functional RPE cells have been derived from hESCs, first via spontaneous differentiation (Klimanskaya et al. 2004; Lund et al. 2006) and then by more rapid and efficient protocols assisted by Nicotinamide and Activin A (Idelson et al. 2009). These RPE cells can be purified by manual picking, which is effective but laborious, or by a simpler enzymatic process (Maruotti et al. 2013).

Recent technological advances have created three dimensional organoid cultures resembling the optic cup or the neural retina. In modified serum-free and growth-factor-reduced medium (SFEBq culture), mESCs spontaneously form a hollow vesicle of neuroepithelium. The suspension organoid cultures then can form a cup-like structure resembling the embryonic optic cup, a process driven by self-organization (Eiraku et al. 2011). Similar self-forming optic cup structures have been observed when using human pluripotent stem cells (Nakano et al. 2012; Zhong et al. 2014). The 3-D organoid cultures result in more robust and efficient retinal cell differentiation and are better models to recapitulate eye development. Still, production of functional photoreceptors with fully developed outer segments *in vitro* remains a key goal.

## 72.2 Using iPSCs to Model Retinal Degenerative Diseases

The regenerative medicine field witnessed another unprecedented discovery when Takahashi and Yamanaka reported the first study on turning somatic cells into an embryonic stem cell-like state: induced pluripotent stem cells (iPSCs) (Takahashi and Yamanaka 2006). Like ESCs, iPSCs can give rise to the full repertoire of somatic cell types. Most importantly, iPSCs match the patient from which they are derived in genetic background, and therefore are invaluable to model diseases, especially those with a strong genetic component. The RPE cells derived from human iPSC lines show similar properties to the ones derived from hESCs: they have similar gene expression profiles and phenotypic features, e.g. they maintain ZO-1 positive tight junctions, express functional visual cycle enzymes and are capable of photoreceptor outer segment (POS) phagocytosis (Buchholz et al. 2009; Meyer et al. 2009; Osakada et al. 2009; Maeda et al. 2013). In addition, neural retinal progenitor cells and their progeny including photoreceptor cells were also successfully derived from human iPSCs (Meyer et al. 2009; Osakada et al. 2009; Lamba et al. 2010; Mellough et al. 2012; Zhong et al. 2014).

Most retinal degenerative diseases are complex, and their underlying mechanisms remain unclear. Disease modeling using patient-specific iPSCs is a promising approach to elucidate the mechanisms of degenerative disorders. Patient-specific iPSC lines derived from RP patients with distinct mutations in the *RP1, RP9, PRPH2* or *RHO* genes have been generated, and rod photoreceptors derived from such lines expressed markers of cellular stress and underwent degeneration, recapitulating key aspects of the disease (Jin et al. 2011). In a separate study, iPSCs derived from RP patients with mutations in the *USH2A* gene were used to generate the multi-layer eyecup-like organoid cultures (Tucker et al. 2013). Analysis of the photoreceptor precursor cells revealed that the USH2A variant Arg4192His causes photoreceptor degeneration through protein mis-folding and ER stress (Tucker et al. 2013). Best disease (BD) is another inherited retinal degenerative disease; it is caused by mutations in the *BESTROPHIN1 (BEST1)* gene. RPE cells derived from BD patient-specific iPSCs are less effective in conducting POS phagocytosis (Singh et al. 2013). These studies demonstrate the potential of using patient-specific iPSCs to model and study retinal degenerative diseases. More such retinal disease models are anticipated, and further studies are eagerly awaited to identity disease pathways and drug candidates.

## 72.3 Direct Cellular Reprogramming

Several cellular reprogramming strategies have been developed: (1) cell fusion; (2) nuclear transfer (3) forced expression of cell fate specific transcription factors; (4) stimulation with small molecules and environmental factors. The iPSC reprogramming technology indicates that surprising plasticity is present in many types of cells. However, it takes multiple steps and a long time to reprogram somatic cells back to a pluripotent state then differentiate them towards the targeted cell types. An alternative is direct reprogramming, which aims to switch cells from one type to another directly. To date, there are just a few studies focused on generating induced retinal cells or induced RPE cells via direct cellular reprogramming.

Cell fusion mediated somatic cell reprogramming is a classic strategy to push cells towards different fates (Ambrosi and Rasmussen 2005). Retinal cells including Müller glia, amacrine and retinal ganglion neurons can fuse with transplanted hematopoietic stem and progenitor cells (HSPCs), ESCs or retinal stem and progenitor cells (RSPCs) *in vivo* upon retinal damage (Sanges et al. 2013). Activation of the Wnt/β-catenin signaling pathway in the transplanted cells is critical for cell fusion and reprogramming to occur. The fused cells can proliferate and differentiate *in vivo*, to partially regenerate the damaged retinal tissue (Sanges et al. 2013).

Müller glial cells are an endogenous resource for regeneration and repair of retinal injuries in fish and amniotes, and several studies have examined the plasticity of mammalian Müller glia. Müller glia harvested from both adult human vitreoretinal explants and the adult mouse retina are able to produce cells similar to other retinal

cell types, including bipolar, amacrine, horizontal cells and photoreceptors, under a defined differentiation environment (Giannelli et al. 2011). Forced-expressing of *Ascl1* (*Mash1*) in mouse Müller glia cells resulted in retinal progenitor-like cells that could proliferate *in vitro* and showed neuron-like response to neurotransmitters (Pollak et al. 2013).

Other cell types also show potential for direct reprogramming into retinal progeny. By forcing expression of the photoreceptor specific homeobox gene *Crx*, primary cells derived from adult rat iris tissue could produce photoreceptor-like cells that expressed rhodopsin and recoverin (Haruta et al. 2001). Combinations of (1) *Crx* and *Otx2*, (2) *Crx, Nrl* and *NeuroD* or (3) *Crx, Rx* and *NeuroD* produced similar results, and generated photoreceptor-like cells that express photoreceptor-specific markers and exhibited rod photoreceptor-specific electrophysiological responses to light stimuli (Akagi et al. 2004, 2005; Seko et al. 2012). A related strategy has been applied to generate RPE-like cells from human fibroblast cells. A combination of *cMYC, Mitf, Otx2, Rax, Crx, Kif4, Nrl* and *Pax6* was found to reprogram human fibroblast cells into RPE-like cells (Zhang et al. 2014). The induced RPE-like cells form a typical cobblestone morphology and express key RPE markers including *Bestrophin1, ZO-1* and *Cralbp* but have low expression of *RPE65* and *Tyr*. It will be useful to perform additional characterization of these RPE-like cells, including examination of cell polarity, physiology and phagocytosis, to understand how similar they are to native RPE.

Specific combinations of intrinsic factors and environmental cues are critical for successful direct reprogramming. Additional work to optimize conditions such as the mixture of transcription factors, the growth factors used, and the sequence of their application, is needed to determine the optimal protocols for deriving specific retinal and RPE cells that function well. Nevertheless, work to date indicates that direct cellular reprogramming is a viable and potentially more efficient strategy to generate specific retinal cell types from various sources of cells.

## 72.4 Future Perspectives

Through these pioneering stem cell studies we have learned that key factors that generate neural retinal and RPE cells are evolutionarily conserved, and that the retinal cells emerging in the dish have remarkable powers of self-assembly to create structures with appropriately organized layers. Still, there is the need for improvements in technologies that will include (a) even more efficient and consistent differentiation protocols, especially for the neural retinal lineages (b) more rapid differentiation, (c) production of purified retinal populations. Using the variety of culture methods being developed, from 2D to organoid cultures, we look forward to gaining a better understanding of human retinal cell development. We predict that iPSC-based modeling will profoundly improve study of disease mechanism and therapeutic development. An exciting future strategy deserving exploration is regeneration of retinal

cells via endogenous sources such as RPE cells and Müller glia. This will require strategies to safely activate the target cells, and possibly direct reprogramming by introducing genes, taking advantage of strides made in viral gene delivery to the retina (Day et al. 2014). In summary, stem cell research provides the opportunity to advance basic research relevant to human retinal development and function. We look forward to translational research progress from bench to bedside, and ultimately, to a new era of regenerative medicine for preserving and improving vision.

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