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Review

# Embryonic stem cells and retinal repair

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**Abstract**

In this review we examine the potential of embryonic stem cells (ESCs) for use in the treatment of retinal diseases involving photoreceptors and retinal pigment epithelium (RPE). We outline the ontogenesis of target retinal cell types (RPE, rods and cones) and discuss how an understanding of developmental processes can inform our manipulation of ESCs *in vitro*. Due to their potential for cellular therapy, special emphasis is placed upon the derivation and culture of human embryonic stem cells (HESCs) and their differentiation towards a retinal phenotype. In terms of achieving this goal, we suggest that much of the success to date reflects permissive *in vitro* environments provided by established protocols for HESC derivation, propagation and neural differentiation. In addition, we summarise key factors that may be important for enhancing efficiency of retinal cell-type derivation from HESCs. The retina is an amenable component of the central nervous system (CNS) and as such, diseases of this structure provide a realistic target for the application of HESC-derived cellular therapy to the CNS. In order to further this goal, the second component of our review focuses on the cellular and molecular cues within retinal environments that may influence the survival and behaviour of transplanted cells. Our analysis considers both the potential barriers to transplant integration in the retina itself together with the remodelling in host visual centres that is known to accompany retinal dystrophy.


*Keywords:* Human embryonic stem cells; Degeneration; Development; Transplantation; RPE; Binucleation; Lens; Lentoid; Photoreceptor; Cone; Opsin; Recoverin; Macular degeneration; Retinitis pigmentosa; Microglia; Müller cells; Astrocyte; Inflammation; Integration; Barrier; Matrix; Immune rejection

## 1. Introduction

The field of embryonic stem cell (ESC) research holds significant potential for the treatment of CNS disorders. The retina is the photosensitive component of the CNS located in the eye. The accessibility of the retina, together with imaging technologies and an extensive knowledge base surrounding the organisation and function of this structure make it a prime candidate for developing cellular therapies for CNS disorders. This point has been highlighted recently by the use of the retina to study development of neural progenitor cells.

Amongst patients that may benefit directly from an ESC-based retinal cellular replacement strategy are those suffering from currently incurable eye diseases such as age-related macular degeneration (AMD) and retinitis pigmentosa (RP). In particular, AMD is the leading cause of blindness in the developed world, with approximately 25 to 30 million people affected worldwide by some form of the disease. Within the UK, the percentage of those afflicted by this condition is close to 1% of the population (figures from the Royal National Institute for Blind People and The Macular Disease Society).

The loss of vision in AMD and RP results from death or irreversible damage to the retinal pigment epithelium (RPE) and/or photoreceptors (Curcio et al., 1996; Delyfer et al., 2004; Young, 1987). However, because the ganglion

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cell output neurons of the retina are largely preserved (Medeiros and Curcio, 2001; Stone et al., 1992), this makes the replacement of photoreceptors and RPE a viable option for restoring visual function in such diseases.

The feasibility of RPE cell replacement in AMD has already been demonstrated by autologous transplantation of full thickness RPE/choroid from periphery to macular (MacLaren et al., 2007; van Meurs and Van Den Biesen, 2003). However, due to the volume of patients with retinal disease, surgical complexity and genetic defects in autologously derived cells, the current review focuses on human embryonic stem cells (HESCs) as a source of material for retinal repair.

A growing body of convergent data indicates that HESCs can give rise to retinal cells following variable degrees of manipulation. In order to more fully understand the mechanisms involved in this process, we examine the developmental cues that specify identity and maintenance of RPE/photoreceptors. We review existing methods for ocular cell derivation from HESCs and highlight how insights gained from the developmental literature are already enhancing the efficiency by which photoreceptor progenitors can be produced.

We argue that the success to date in deriving ocular cell types from HESCs may be at least in part due to the established protocols for derivation, propagation and neural differentiation of these cells. Finally, we address key issues surrounding the introduction of HESC-derived RPE and photoreceptors into a diseased retinal environment. In particular, we highlight the challenges of immune rejection, barriers to cellular integration and neuronal plasticity, which should be considered by future investigations in the field.

## 2. Developmental cues for specification of ocular tissue

After an overview of ocular development we focus on molecular cues relevant to the derivation of photoreceptor progenitors (specifically cones) and RPE cells. For more detailed reviews of retinal development and cell-type specification please see reviews by Cepko, Harada and Klassen (Cepko et al., 1996; Harada et al., 2007; Klassen et al., 2004a).

### 2.1. Overview of ocular development

In vertebrates, neural tissue is defined during the gastrula stage, a process thought to involve antagonism of bone morphogenetic protein (BMP) and Wnt signalling pathways (Glinka et al., 1998; Munoz-Sanjuan and Brivanlou, 2002). With regard to the present review, two of these molecules are of particular note, the BMP antagonist noggin and the Wnt antagonist Dickkopf-1 (*Dkk*1).

The antagonism of Wnt signalling during forebrain development helps to define telencephalon and eyefields, with specification of the latter structures involving a hierarchical network of eye field transcription factors (EFTFs) that may be directed by Otx2 and Rx (Stigloher et al., 2006; Zuber et al., 2003). Other components to consider here are the insulin-like growth factor (IGF) signalling pathway, which is implicated in eye field induction (Pera et al., 2001) and Notch signalling which has been shown to induce EFTF expression and ectopic eye tissue formation (Onuma et al., 2002).

Following eye field specification, optic vesicles arise bilaterally from the forebrain and expand into mesodermal tissue of the head. Inductive interactions ensue between the optic vesicles and a thickened portion of overlying surface ectoderm called the lens placode. These interactions lead to invagination of optic vesicles and the formation of optic cup and lens vesicle (Pei and Rhodin, 1970).

Once formed, the neuroectodermal tissue of the optic cup gives rise to several spatially demarcated structures: neural retina, RPE and, at its far periphery, the epithelium of the iris and ciliary body (Beebe, 1986). The RPE develops adjacent to the choroid, a structure composed of cells derived from both cranial neural crest and mesenchyme (Etchevers et al., 2001; Noden, 1982; Torczynski, 1982).

During human eye development, pigmentation is first observed in the external layer of the optic cup (the newly differentiating RPE cells) some time between post-ovulatory weeks 5 and 6 (O'Rahilly, 1966, 1975). To date, most of our understanding about mammalian RPE development comes from studies in rodents. It is evident from these studies that RPE cells achieve near maximal pigmentation levels early in the developmental process and that they undergo two clear phases of mitosis (Stroeva and Mitashov, 1983). Interestingly, the second phase of mitosis lacks cytokinesis, a phenomenon resulting in the majority of adult rat RPE cells being binucleated. This is a much neglected and highly conserved feature of vertebrate RPE development with multinucleated cells also commonly observed in human RPE (Han et al., 2006; Stroeva and Mitashov, 1983).

### 2.2. The neural retina and retinal pigment epithelium

The key cellular decision to be made within the developing optic cup is that of commitment to neuroretinal versus RPE cell fate. In large part, this choice is determined by proximity of neuroepithelial optic cup cells to signals emanating from surrounding extraocular tissues (Fuhrmann et al., 2000; Moshiri et al., 2004). The proximal optic vesicle is exposed to factors from the surrounding head mesenchyme that convert cells in this location into RPE, while cells positioned towards the distal aspect become neural retina in response to signals from surface ectoderm.

The RPE-genesis signal is thought to derive from TGFβ superfamily signalling molecules such as BMP and/or activin (Moshiri et al., 2004). In this respect, the extraocular mesenchyme has been identified as the key source of RPE-genesis signal in developing chick eyes (Fuhrmann et al., 2000). In this study, Fuhrmann and colleagues clearly demonstrated that activin A could successfully sub-