Computational and Structural Biotechnology Journal 13 (2015) 382–389

COMPUTATIONAL AND STRUCTURAL BIOTECHNOLOGY JOURNAL

journal homepage: www.elsevier.com/locate/csbj

Mini Review

Using Stem Cells to Model Diseases of the Outer Retina

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ARTICLE INFO

Article history:
Received 22 January 2015
Received in revised form 30 April 2015
Accepted 1 May 2015
Available online 6 May 2015

Keywords:
Disease models
Induced pluripotent stem cells
Retinitis pigmentosa
Age related macular degeneration
Leber congenital amaurosis
Inherited retinopathy

ABSTRACT

Retinal degeneration arises from the loss of photoreceptors or retinal pigment epithelium (RPE). It is one of the leading causes of irreversible blindness worldwide with limited effective treatment options. Generation of induced pluripotent stem cell (IPSC)-derived retinal cells and tissues from individuals with retinal degeneration is a rapidly evolving technology that holds a great potential for its use in disease modelling. IPSCs provide an ideal platform to investigate normal and pathological retinogenesis, but also deliver a valuable source of retinal cell types for drug screening and cell therapy. In this review, we will provide some examples of the ways in which IPSCs have been used to model diseases of the outer retina including retinitis pigmentosa (RP), Usher syndrome (USH), Leber congenital amaurosis (LCA), gyrate atrophy (GA), juvenile neuronal ceroid lipofuscinosis (NCL), Best vitelliform macular dystrophy (BVMD) and age related macular degeneration (AMD).

Contents

1. Introduction

Retinal degeneration is one of the leading causes of irreversible blindness worldwide with limited effective treatment options. Retinal degeneration is the end point of many differing disease processes. In 2014, inherited retinopathies overtook diabetic related causes for blind registration in the working population in the UK [1] and in older individuals the major cause for sight loss is age related macular degeneration (AMD) [2] with over 40 million sufferers worldwide. The retina is located in the posterior chamber of the eye, lining its inner surface and comprises multiple layers of differing cell types. The majority of primary retinopathies affect the outer retina, which is primarily formed of the photoreceptors and its monolayer of support cells termed the retinal

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http://dx.doi.org/10.1016/j.csbj.2015.05.001

pigment epithelium (RPE) [3]. Inherited retinopathies can affect the development of the light-sensitive photoreceptor cells of the retina, the function of retinal and RPE cells, or can result in the premature loss of these cells (Fig. 1). As the intrinsic regenerative capacity of the retina and RPE is limited, one potential therapy is cellular replacement [4].

Over the past decade, stem or progenitor cell transplantation as a means of replacing tissue has evolved rapidly, with the development of protocols to drive embryonic stem cells (ESCs) towards the fate of both photoreceptors or the RPE [6] [5–15] Despite this, regenerative therapy is only in the early stages of clinical trial and is currently being developed primarily for the more common retinal degenerations and each disease poses its own challenges. Therefore, in order to learn more about these wide ranging pathologies, there is a need to develop robust models to target therapies. Until recently in retinal biology, animals have been used primarily to model disease. While this has benefits in examining an organ in relation to an organism, there are major drawbacks in terms of increasing ethical objection to the use of animals in research and critical differences between human and animal species, for example the absence of a macular region in the common rodent animal models.

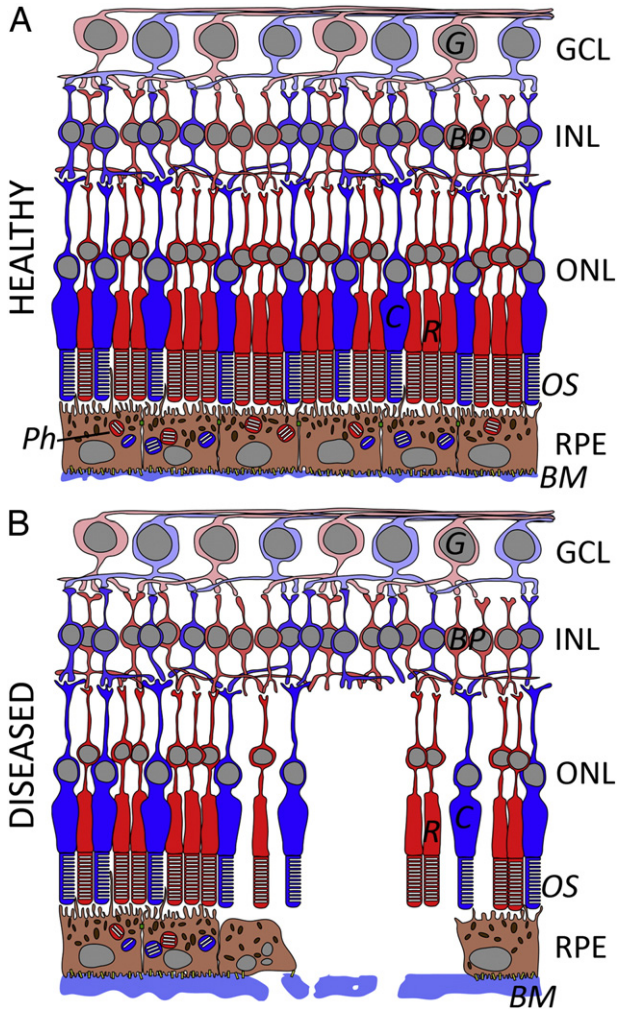

Fig. 1. A diagrammatic comparison of healthy (A) and diseased (B) retina highlighting the strong interdependence between photoreceptors and RPE. In the diseased retina, a decreased number of cones (C) and rods (R) is associated with RPE cell loss. This may lead to RPE detachment, where the RPE is lifted off the Bruch's membrane (BM) overlying the choroid. In addition, there is a reduction of phagosomes (Ph) in the RPE, as well as decreased phagocytosis of POS. In contrast, the neural circuits comprising bipolar cells (BP) and ganglion cells (G) remain comparatively unchanged. INL inner nuclear layer; ONL outer nuclear layer; GCL = ganglion cell layer. Adapted from Ramsden *et al.* [5].

In 2006, a major advance in stem cell technology produced a new means with which to investigate inherited diseases, such as retinopathies, in diseased patient cells *in vitro*. In a phenomenal series of experiments, Takahashi and Yamanaka identified the embryonic transcription factors that are required to turn an adult somatic cell into a pluripotent stem cell. The group was able to reprogram mouse [16] and subsequently human fibroblasts [17] into stem cells, termed induced pluripotent stem cells (IPSCs) using retroviral transduction with the four transcription factors OCT4, SOX2, KLF4 and c-MYC. Using this technology, fibroblast cells, which are readily accessible in the form of a skin biopsy, can be taken from sufferers of retinal diseases and converted into IPSCs. IPSC-derived eye cells will provide us with a new platform to investigate diseases in cell types, which have previously been inaccessible. In this article, we explore the current state of published outer retinal disease models using IPSCs and the technical difficulties encountered in their generation.

2. Technical Challenges for IPS Generation, Differentiation and Use

IPSCs can be generated from a wide array of sources including fibroblasts, keratinocytes, and T cells. The cell source can contribute to the epigenetic memory and serves as a challenge for IPSC research. Numerous studies have highlighted the varied growth and differentiation characteristics of IPSC lines. This is thought to be caused by a combination of genetic and epigenetic variation leading to subtle but significant differences in endogenous signalling. [18–21] Therefore, recent methods for deriving IPSCs from somatic cells may not always yield uniform lineage competencies between lines [40]. This necessitates optimization of IPSC-retinal cell differentiation protocols to suit each IPSC line. This fact, in combination with the long time scales and low efficiency of the majority of differentiation protocols makes for a long and expensive period of research and development before a reliable method for generating retinal cells from IPSCs can be established.

Patient IPSC-derived cells/tissues can then be used to span a range of applications from elucidating the mechanism of disease causing mutations to drug and gene therapy testing (Fig. 2). The experimental process requires that IPSC lines are also generated from healthy control individuals in order to measure differences in the mutation carrying patient cells [50]. An alternative strategy is to generate isogenic control cell lines where the mutation of interest is corrected in patient cells using gene editing technology. Control and test cell lines must then be differentiated simultaneously into the target cell. In order to make valid comparisons, it is ideal that the identity and maturity of the cells derived from both test and control cell lines are equal. Extensive studies of ESC lines has shown differences in their innate differentiation propensity [15,22]; thus it is likely that subjecting two cell lines to the same protocol could generate a different array of cell types. This could become problematic if retinal cells are identified and isolated from differentiated stem cell cultures based on the expression of markers that are also expressed in a range of neuronal cell types (*e.g.* Pax6, Chx10). Similarly, markers that are specific to a level of cell maturity are important. For example Recoverin is expressed early in human retinal development [23] and persists thereafter; therefore, using Recoverin expression as the *de facto* inclusion criteria for IPSC derived photoreceptors would include a range of cell types from developmentally immature progenitors to photoreceptors. If these potential pitfalls are not properly accounted for, incorrect conclusions about disease aetiology may be drawn.

The pure population of differentiated cells often has a limited proliferative capacity necessitating continued derivation from the original pluripotent IPSC bank [24]. IPSCs may incur mutations and chromosomal loss over time in culture as well as a secondary shortening of their telomere and reduced cell growth making the diligent maintenance of the cell bank crucial [26,27].

Thus far IPSCs have been used to generate several cell types that are implicated in retinal degenerative diseases, including RPE [28], retinal