A revolution in sight: using induced pluripotent stem cells to investigate diseases of the retinal pigment epithelium

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Macular diseases are one of the leading causes of vision loss in the western world. In the UK alone, almost 1.5 million people suffer from these devastating diseases, which primarily affect the macula, a region in the retina responsible for detailed central vision. In many patients, cellular changes, attributable to ageing or inherited mutations, are associated with the retinal pigment epithelium (RPE), a single layer of cells that sustains and supports the light-sensitive retina. In the absence of functional RPE, the retina becomes damaged and vision deteriorates. Currently, there are no treatments for these diseases. Over the last two decades, induced pluripotent stem cells have revolutionized our study of retinal disorders, enabling researchers to produce previously inaccessible RPE cells in a dish. The ability to recreate these cells from patients has provided new model systems to understand the mechanisms behind the disease and accelerate the development of new therapies to treat sight loss.

The retinal pigment epithelium

Our eyes are complex organs. In the retina alone, there are over 60 distinct cell types responsible for detecting and converting light into signals that are conveyed to the brain to form an image. The outermost layer of the retina is composed of cells called the retinal pigment epithelium (RPE), which help maintain and promote the health and function of the neural retina. The RPE forms a continuous tight pigmented monolayer with a distinct black cobblestone-like appearance that acts as a gatekeeper. These cells form an integral part of the blood:retina barrier, separating the choroidal blood supply from the unique ionic environment of the light-sensing retina required for photoreceptor excitability (Figure 1a). To maintain retinal homeostasis, the RPE performs several vital roles (Figure 1b), mediating the selective movement of ions, nutrients, molecules and water to and from the retina, phagocytosing the outer segment debris shed daily from the photoreceptor cells and recycling retinal to replenish the visual cycle. The RPE secretes growth factors and cytokines, helping to maintain immune privilege in the eye, and contains melanosomes that absorb stray light, protecting the retina from oxidative stress.

The human retina is composed of rod photoreceptor cells, sensitive to low levels of light, and cone photoreceptors, responsible for colour vision in bright conditions. To enable high acuity vision, such as reading, driving and recognizing faces, cones are concentrated in a central area of the retina, known as the macula. This area contains the highest density of photoreceptors and RPE in the retina, with each RPE cell estimated to look after 20 individual photoreceptors. As one of the most metabolically active areas of the body, the macula relies on the RPE to sustain the crucial activities of the photoreceptors responsible for central vision. In humans, both the retina and the RPE terminally differentiate during development and have no regenerative capabilities; this means that the cells we are born with must maintain good function to support the retina. However, over a lifetime, the burden of looking after the retina can cause the build-up of debris and toxic products, such as lipofuscin, in the RPE, resulting in dysfunction or cell death and ultimately leading to the subsequent death of overlying photoreceptors. Injury or loss of RPE is an important underlying cause of degenerative and inherited forms of blindness.

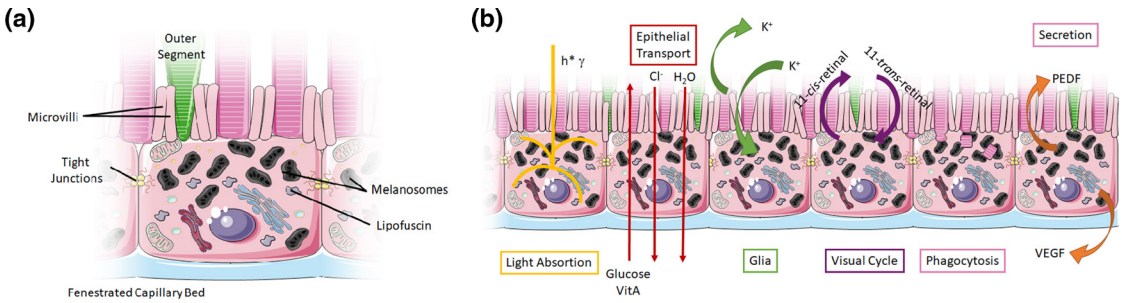

Figure 1. Retinal pigmented epithelium. (a) The polarized retinal pigment epithelium interacts with the outer segments of the photoreceptor cells and (b) performs crucial roles to maintain the retina (based on Strauss, 2005; designed using Servicer Medical ART).

The retinal pigment epithelium and macular disease

There are a variety of macular diseases that cause RPE dysfunction (Table 1), the most prevalent being age-related macular degeneration (AMD), a multifactorial disease that is the leading cause of blindness in patients over 60 years in the western world. The biggest risk for developing AMD is age; however, smoking, obesity, cardiovascular disease, ethnic origin and genetic background can increase the risk of developing AMD. Within the macula, at the interface between the RPE and the vascular layer, the accumulation of yellow fatty deposits, termed 'drusen', disrupts the RPE's structure and function. Over time, disease progression initiates a cascade of RPE and retinal cell death, leading to the slow loss of vision, known as dry (geographic) AMD. In approximately 10% of cases, AMD can be diagnosed as a wet (neovascular) form. Wet AMD occurs when the RPE barrier is disrupted, leading to the growth of new blood vessels from the choroidal blood supply towards the retina. These leaky vessels allow blood cells and fluid to enter the retina, immediately disrupting vision and leading to photoreceptors loss in the long term. The progression of wet AMD can be delayed by monthly injections of anti-VEGF drugs, which help to stabilize vision by slowing or stopping abnormal blood vessel growth. However, there are no treatments currently available for patients, with 90% of patients having dry AMD.

Inherited forms of macular disease can be caused by mutations in genes crucial to RPE function. For example, Best disease is a macular dystrophy caused by mutations in the *BEST1* gene; *RPE65* mutations can result in Leber congenital amaurosis or retinitis pigmentosa; late-onset retinal degeneration is a consequence of *C1QTNF5* mutations; Sorsby fundus dystrophy is caused by mutations in *TIMP3*; and Doyne honeycomb retinal dystrophy develops due to mutations in *EFEMP1*. In addition, the RPE can also be affected by mutated genes expressed by other cells, e.g., mutations in *ABCA4*, a protein expressed in the outer segments of photoreceptor cells, cause the accumulation of toxic compounds in the RPE (see Table 1).

Table 1. Macular diseases resulting from dysfunction or degeneration of the retinal pigment epithelium (data from OrphanNet)

Disease	Cause	Prevalence	Onset
Age-related macular degeneration	Multiple genetic and environmental risk factors	1 in 8 (60+ years)	50+ years
Best	BEST1 – ion channel	1 in 5000	Variable
RPE65 Leber congenital amaurosis	RPE65 – visual cycle enzyme	1 in 80,000	Childhood
Retinitis pigmentosa	RPE65 – visual cycle enzyme MERTK – phagocytosis	1 in 3000	Variable
Late-onset retinal degeneration	C1QTNF5 – cell adhesion	<1 in 200,000	50+ years
Sorsby fundus dystrophy	TIMP3 – regulation of extracellular matrix	1 in 220,000	40+ years
Doyne honeycomb retinal dystrophy	EFEMP1 – extracellular matrix protein	<1 in 200,00	40+ years
Stargardt disease	ABCA4 – membrane transporter	1 in 10,000	Childhood

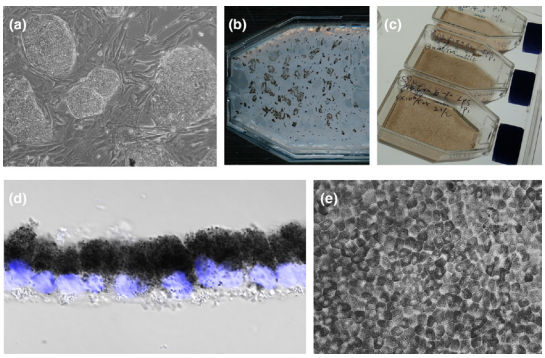

Figure 2. Production of RPE from IPSCs. (a) Patient-derived IPSCs cells grow as distinct colonies. (b) Spontaneous differentiation of IPSCs into pigmented RPE patches. (c) Pigmented patches can be isolated, dissociated and plated to form pigmented RPE monolayers in culture. (d) A cross-section through an IPSC-RPE monolayer, showing pigment granules and nucleus (blue). (e) The classic epithelial cobblestone appearance of IPSC-RPE.

Investigating RPE disease using pluripotent stem cells

For many macular diseases, the build-up of toxins, debris and fluids in and around the RPE is a marker of late-stage disease. Identifying the initial pathological events that lead to macular degeneration could allow researchers to understand the early mechanisms behind disease development and discover targetable pathways for future therapeutic development. In the past, this process has been difficult, hampered by (1) animal models that do not accurately reflect human diseases, (2) RPE cell lines, which undergo epithelial–mesenchymal transition in prolonged culture or (3) post-mortem tissues, which are in limited supply and rarely from early-stage disease. However, our ability to model macular diseases in a dish was revolutionized in 2006, when Nobel Prize winner Shinya Yamanaka first discovered a method to reprogram adult cells, such as skin fibroblasts, into pluripotent stem cells using four key embryonic transcription factors, Oct3/4, Sox2, Klf4 and c-Myc. These so-called induced pluripotent stem cells (IPSC) are capable of self-renewal and differentiation into any cell of the body, including the RPE. IPSCs can be created from patients and are a valuable resource to study human disease in previously inaccessible cells (Figure 2a). Patient-derived IPSCs can be guided to differentiate into specific cell types, such as the RPE, generating an unlimited resource of disease-relevant cells for studies.

Our group at the UCL Institute of Ophthalmology has been using IPSCs to generate RPE cells to understand and develop treatments for macular diseases. Compared to other cell types of the body, working with IPSC-derived RPE is relatively straightforward, due to the formation of dark brown cells that are easily identifiable in stem cell cultures (Figure 2b). These cells can be manually isolated to form a highly pigmented and polarized cobblestone RPE monolayer, like that found at the back of the eye (Figure 2c–e). In a cell culture dish, IPSC-RPE express proteins normally found in RPE, form a tight barrier and perform functions crucial to the role of the RPE in the eye, including phagocytosis, transport, retinoid processing and factor secretion. Our initial work has focused on developing new models of macular disease to investigate how single-gene mutations affect the RPE (Figure 3). These studies have revealed important changes in protein expression and cell behaviour in patient-derived cells. We are also using IPSC-RPE cells as a platform to test new molecular therapeutics, e.g., testing translation read-through drugs to overcome mutations that induce premature stop codons and optimizing gene therapy to restore protein levels when mutations cause a reduction or absence of protein expression. The holy grail for inherited diseases is to get

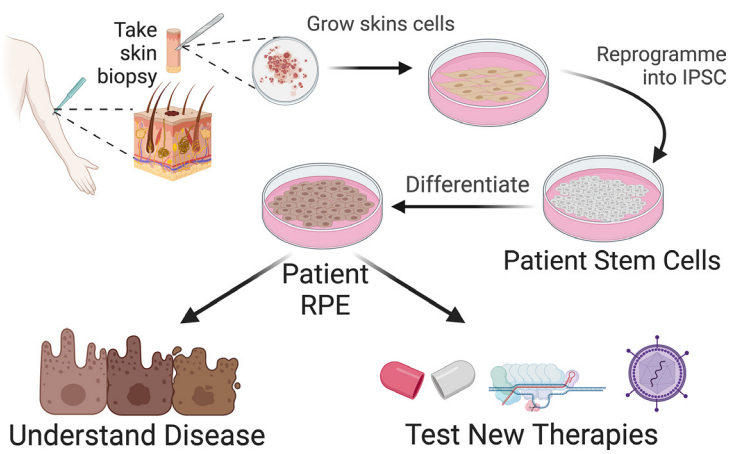

Figure 3. RPE cells can be produced from patient-derived IPSCs to help understand macular disease and test new therapeutics. (Image created with BioRender.com).

to the heart of the problem by fixing the mutated gene itself. CRISPR genome engineering offers a new means to edit patient mutations in a precise manner. However, as a terminally differentiated cell, the options for editing RPE cells may be limited since the DNA repair mechanisms required to replace mutated genes are inefficient in non-diving cells such as those found in the retina. Patient-derived IPSC-RPE will be a crucial platform to test and optimize CRISPR editing and precision-based medicine approaches to treat inherited forms of macular disease.

Studying more complex RPE diseases, such as AMD, presents several challenges. The largest risk factor for AMD is age, and IPSC-RPE are more similar to embryonic RPE. Researchers therefore need to identify methods to imitate or accelerate the process of ageing in IPSC-RPE cells. Many environmental factors, such as high blood pressure, a diet high in saturated fats or smoking, increase the risk of AMD. Will it be possible to mimic the effects of these individually or combined in a dish? Furthermore, there are more than 50 risk variants in the genome that influence susceptibility to AMD. It will be important to understand how combinations of these risk variants contribute to disease pathology in individual patient's IPSC-RPE. Indeed, research using IPSC-RPE created from patients with high- and low-risk AMD variants for complement factor H (an AMD risk gene involved in inflammation) is encouraging. These cells recapitulate many expected features of AMD compared to control cells, including increased cell stress, inflammation, fatty compound accumulation and drusen-like deposits.

Conclusion

Due to their contribution to sight loss, RPE cells are a key focus for the development of treatments for macular diseases. Using patient-derived IPSCs to create RPE provides researchers with an unlimited source of cells to perform *in vitro* studies of inherited and degenerative diseases, which may lead to the identification of new biological targets intrinsically associated with early pathological events. These cells can be used as an essential platform to assess the new era of precision and genomic-based medicines, ensuring the effectiveness and safety of these approaches in diseased cells in a dish, ultimately advancing their translation towards clinical trials. Looking to the future, differentiation of other cell types from IPSCs (e.g., photoreceptors with outer segments, endothelial and immune cells) could allow us to co-culture RPE with other relevant cell types to examine disease-associated interactions in an environment more representative of the back of the eye. IPSC-RPE are also being developed for regenerative medicine approaches to replace cells lost because of disease. Theoretically, this could lead to each patient being treated with IPSC-RPE created from their own cells (autologous transplant) or the creation of IPSC biobanks, where patients can be matched with compatible donor cells (allogenic transplant). In 2005, the *Science* edition, '125 questions: what we don't know?', predicted that reprogramming skin cells into stem cells and guiding them to form tissues would be worth 'more than its weight in gold'. Less than 20 years later, we are now at the stage where this cellular alchemy is a reality and is transforming our understanding and treatment of human vision loss. ■

Website

https://www.ucl.ac.uk/ioo/research/research-labs-and-groups/carr-lab/bestrophinopathies-resource-pages

Further reading

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