Chapter XX

Bestrophin1: A gene that causes many diseases

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Abstract Bestrophinopathies are a group of clinically distinct inherited retinal dystrophies that lead to the gradual loss of vision in and around the macular area. There are no treatments for patients suffering from bestrophinopathies and no measures can be taken to prevent visual deterioration in those who have inherited disease-causing mutations. Bestrophinopathies are caused by mutations in the Bestrophin 1 gene (BEST1), a protein found exclusively in the retinal pigment epithelial (RPE) cells of the eye. Mutations in BEST1 affect the function of the RPE leading to the death of overlying retinal cells and subsequent vision loss. The pathogenic mechanisms arising from BEST1 mutations are still not fully understood, and it is not clear how mutations in BEST1 lead to diseases with distinct clinical features. This chapter discusses BEST1, the use of model systems to investigate the effects of mutations and the potential to investigate individual bestrophinopathies using induced pluripotent stem cells.

X.X Introduction

Bestrophinopathies are a collection of inherited retinal diseases caused by mutations in Bestrophin1 (BEST1). These diseases, including Best disease, adult onset vitelliform macular degeneration (AVMD), autosomal recessive bestrophinopathy (ARB), autosomal dominant vitreoretinchoroidopathy (ADVIRC) and autosomal dominant retinitis pigmentosa (adRP), have varying times of onset and significantly different clinical manifestations (Table 1; For review see Leroy, 2012). Diagnosis of a bestrophinopathy is usually confirmed by an abnormal light-to-dark ratio in the electrooculogram (EOG), a test that assesses the standing potential at the back of the eye; an indirect measurement of retinal pigment epithelium (RPE) cell function. This pathognomonic test, together with protein localisation, hyperpigmentation, the accumulation of fluid and autofluorescent material within and around the macula, suggests that the origin of BEST1-related diseases resides within the RPE.
**X.X RPE**

The RPE is a tight barrier of cells that resides as a pigmented cobblestone-like monolayer on Bruch’s membrane, between the neural retinal and the choriocapillaris, where it forms a crucial component of the blood retina barrier. The RPE is involved in a wide range of functions essential for the maintenance of the neural retina, including the daily phagocytosis of shed photoreceptor outer segments, recycling of retinol in the visual cycle, secretion of signalling molecules and growth factors, absorbance of stray light and the transport of water, ions, metabolites and nutrients, to and from the retina (For review see Strauss, 2005).

**X.X Bestrophin1**

Bestrophin1 was first identified as the gene responsible for Best disease in the 1990’s. Genetic linkage analysis mapped the gene to chromosome 11q13 (Stone et al. 1992) and in 1998, the gene responsible, termed *vitelliform macular degeneration 2 (VMD2)* or *Bestrophin1 (BEST1)* was identified (Petrukhin et al. 1998). The *BEST1* gene spans 11 exons and produces a 585a.a. protein with a molecular weight of 68kDa. Approximately 300 mutations in *BEST1* have been identified, with allelic heterogeneity leading to the variety in phenotypes associated with bestrophinopathies.

Bacterial and chicken BEST1 proteins provide structural models suggesting a highly conserved N-terminus containing four transmembrane domains and a long, diverse cytosolic portion that contribute to the formation of an ion channel (Dickson et al. 2014; Yang et al. 2014). Five BEST1 protein subunits form a pentameric channel with a small extracellular region and larger intracellular region. The five subunits are symmetrically arranged round a central axis to produce a barrel shaped pore with a hydrophobic neck. BEST1 acts as a Ca$^{2+}$-activated Cl$^{-}$ channel; the binding of Ca$^{2+}$ to the intracellular Ca$^{2+}$ clamps of the BEST1 subunits is associated with dilation of the gate and flux of Cl$^{-}$ ions. The majority of disease-causing mutations reside
in hot spots of conserved residues within the N-terminal portion of BEST1, with many mutations, localised to the Ca\(^{2+}\) clasp and hydrophobic neck, thought to alter the permeability of the channel. Various studies have shown that BEST1 acts as a Ca\(^{2+}\)-activated Cl\(^-\) channel (Soria et al. 2009). It may also act as a volume-activated anion channel (Fischmeister and Hartzell, 2005), and be involved in the regulation of voltage-gated Ca\(^{2+}\) channels (Yu et al. 2006), the recruitment of Ca\(^{2+}\) from endoplasmic reticulum stores (Neussert et al. 2010), intracellular trafficking (Milenkovic et al. 2011) and the mediation of neurotransmitter release (Woo et al. 2012).

**X.X Animal Models of Bestrophinopathies**

In the eye, BEST1 is expressed in the RPE cells, where it is localised to the basolateral membrane (Marmorstein et al. 2000). Its role in the development of human RPE cell pathology has remained elusive as post-mortem tissues are often at end stage disease and reveal little of the molecular sequelae leading to vision loss. Animal models of bestrophinopathies have provided some insights into the disease. Mouse and rat models of Best disease, where mutated BEST1 proteins have been introduced to the eye using adenovirus, have subretinal deposits and altered light peak electroretinogram responses, indicative of defects in the Cl\(^-\)-induced hyperpolarization of the RPE basal membrane (Marmorstein et al. 2009). However, these animals lack a macula so may not fully represent the full spectrum of human bestrophinopathies. One of the most informative animal models of bestrophinopathy is canine multifocal retinopathy (cmr), an autosomal recessive retinal disorder observed in multiple breeds, caused by naturally occurring cBEST1 mutations. Although dogs do not have a macula, there is a fovea like region containing a high concentration of cones, known as the area centralis. The presence of multiple lesions in the majority of cmr cases is similar to that observed in the recessive human bestrophinopathy, ARB, where homozygous or compound
heterozygous mutations in cBEST1 abolish channel activity resulting in a null phenotype (Guziewicz et al. 2011). Canine models of bestrophinopathy are helping to further our understanding of disease progression and deposit formation in bestrophinopathies, and suggest that abnormal apical microvilli in diseased retinas weaken the interaction between the RPE and overlying photoreceptor cells. Canine models are also aiding in the development of BEST1 AAV gene therapy, which reverses disease induced retina-RPE microdetachments in the canine retina (Guziewicz et al. 2018).

**X.X Induced Pluripotent Stem cells**

Understanding the precise molecular sequelae of full range of bestrophinopathies is key to developing effective therapeutics, cellular therapies and personalised medicines for these diseases. Culture models, such as RPE and non-RPE epithelial cell lines, have been used to investigate RPE function, however issues with transdifferentiation of cells and incorrect polarisation of cells limit their use. Stem cell research is now revolutionising what we know about eye diseases, with induced pluripotent stem cells (iPSC) providing researchers with new means to investigate human blinding diseases in a dish. In 2007, a pivotal study demonstrated that human adult skin cells could be reprogrammed to a pluripotent state using a defined combination of embryonic transcription factors (Takahashi et al. 2007). As iPSCs can be generated from skin/blood/urine cells of patients and then differentiated into specific cell types, this technology offers the unique opportunity of studying genetic mutations in a patient’s own cells. These cells provide a direct link between clinical disease and changes at the molecular and cellular level, and can be used as a platform to develop and test novel therapies (Figure. 1). iPSCs are particularly amenable to the study of ophthalmic diseases originating in the RPE due to the relative ease with which these cells can be differentiated and purified from iPSCs (Carr et al. 2009). iPSC-derived RPE can be cultured as a pigmented cobblestone monolayer
of cells, which express key markers of developing and mature RPE including and possess many of the key physiological aspects of native RPE e.g. transepithelial resistance, phagocytosis of retinal debris, secretion of growth factors, the presence of functioning voltage-gated ion transporters and the mobilisation of intracellular Ca\textsuperscript{2+} in response to ATP (Carr et al. 2009; Kokkinaki et al. 2011; Vaajasaari et al. 2011).

**X.X Investigating bestrophinopathies using iPSC-RPE**

Stem cell studies, using iPSC-RPE derived from bestrophinopathy patients, have revealed a number of pathways associated with RPE cell functions compromised by *BEST1* mutations. The phagocytosis of photoreceptor outer segment (POS) membrane discs is a vital process performed daily by the RPE to maintain the retina. This process is disrupted in best disease iPSC-RPE, with delays in both the internalisation and degradation of POS by the RPE (Marmorstein et al. 2018; Singh et al. 2013). Prolonged exposure to POS leads to the build-up of autofluorescent material and increased oxidative stress in cells (Singh et al. 2013). Ion flow is also affected in patient cells. In WT iPSC-RPE, stimulation with calcium elicits a decrease in intracellular Cl\textsuperscript{−}, this response is absent in cells from best disease patients (Moshfegh et al. 2016). Severely reduced Cl\textsuperscript{−} currents also suggest that BEST1 may function as a volume regulated ion channel regulating cell volume (Milenkovic et al. 2015). Epithelial cell water transport is often coupled to ion transport, failure of the Cl\textsuperscript{−} driven transport of water along with defects in phagocytosis could promote the build-up of extracellular fluid and material in the subretinal space, reducing retinal-RPE adhesion, ultimately contributing to pathological issues such as the vitelliform lesion and macular oedema.

Patient iPSC-RPE are also helping to reveal details of BEST1 channel activators. The Best disease p.I201T mutation, which resides within a proposed ATP binding motif, decreases the binding of ATP and activation of BEST1 in iPSC-RPE, suggesting the presence of an ATP
dependent mechanism regulating BEST1 channel gating (Zhang et al. 2018). Intriguingly, ATP released by photoreceptor cells has been postulated to be the light peak substance observed in the electrooculogram, a test normally used to diagnose bestrophinopathies. Defects in ATP responses could be the determinant behind EOG changes in patients with BEST1 mutations. Given the varying clinical pathology of bestrophinopathies, iPSC-RPE may be important in defining the mechanisms behind discrete molecular pathologies underlying these disparate diseases. The pathology behind these diseases could be accounted for by the effects of the mutation on channel formation, protein function or protein localisation. For example, Best disease and ADVIRC both result from dominant BEST1 mutations, however, while Best disease affects the macula, ADVIRC has widespread effects in the peripheral retina and affects eye development. ADVIRC was predicted to result from aberrant splicing of BEST1, however, normal splicing occurs in ADVIRC iPSC-RPE with the V235A mutation, instead protein was observed at both the apical and basal membrane of cells (Carter et al. 2016), which suggests that mislocalisation across the membrane may play a role in this disease. The six known ADVIRC mutations are clustered in the cytosolic regions of the second and third transmembrane domains of BEST1, which suggests they may affect a critical localisation and/or functional domain.

**X.X Conclusions**

Induced pluripotent stem cells offer a powerful alternative model to investigate inherited diseases such as bestrophinopathies by creating diseased human RPE cells in a dish that recapitulate the spectrum of BEST1-related diseases observed in human patients. Studies using patient derived iPSC-RPE will compliment on-going studies in alternative models to fully understand the role of BEST1 in RPE cells and provide vital insight into the contribution of
BEST1 mutations to individual bestrophinopathies, assisting in the development of disease specific therapies.

References


Marmorstein AD, Marmorstein LY, Rayborn M et al (2000) Bestrophin, the product of the


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<thead>
<tr>
<th>Disease</th>
<th>Symptoms</th>
<th>Inheritance - Prevalence</th>
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<tr>
<td>Best disease</td>
<td>Egg yolk-like vitelliform lesion to scrambled egg-like cyst stage, layering of subretinal material. RPE atrophy and loss of central vision loss. Abnormal EOG, CNV hyperautofluorescence. Early onset, some affected asymptomatic.</td>
<td>Autosomal dominant - 1-9/100,000</td>
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<td>ARB</td>
<td>Diffuse RPE alterations, punctate subretinal deposits, retinal oedema, hyperautofluorescence, RPE atrophy, CNV. Abnormal EOG, central vision loss, early onset.</td>
<td>Autosomal recessive - 1/1,000,000</td>
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<tr>
<td>ADVIRC</td>
<td>Peripheral circumferential hyperpigmentation, white punctate opacities, RPE atrophy, CNV. Developmental abnormalities: microcornea, nanophthalmos, vitreous fibrillar condensation, angle-closure glaucoma, cataract optic nerve dysplasia. Visual field loss. Early onset.</td>
<td>Autosomal dominant - 1/1,000,000</td>
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<td>AVMD</td>
<td>Similar to Best disease with late adult onset and slower progression. Subnormal EOG, mild central vision loss. Can develop CNV and RPE atrophy.</td>
<td>Autosomal dominant(?) - Unknown</td>
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<tr>
<td>adRP</td>
<td>Peripheral retinopathy, hyperpigmentation in peripheral retina, macula oedema. Abnormal EOG and constricted visual field loss. adRP (RP50) could be misdiagnosed</td>
<td>Autosomal Dominant - Unknown</td>
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Table 1. Symptoms associated with clinically distinct bestrophinopathies.