The quest of vision: retina’s daily challenges

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The retina is a complex biological structure located at the back of the eye. Every day, it continually performs an intricate set of tasks to provide us with the sense of vision. The neuroretina encompasses neuronal type of cells including the light-sensitive photoreceptors, which sense the incoming light and trigger the conversion of the visual stimulus information to a neural response relayed to our brain where images are created. This article focuses on the physiological processes occurring in the retina and the exquisite interplay between photoreceptors and the adjacent cell monolayer called the retinal pigment epithelium, which underpins the key visual processes of phototransduction, visual cycle and phagocytosis of spent photoreceptors’ outer segments. We also present examples of functional defects in the retina and how they lead to impaired vision or blindness, and discuss some emerging treatment options for retinal diseases.

How do humans interpret the visual information from their environment? We all know that light passes into our eyes, and that this information is somehow sent to our brain to create our vision – arguably the most intimate of senses. But what exactly happens in the eye? Intriguingly, in vertebrates, light passes through many ocular structures and tissues from the anterior cornea, lens and vitreous before finally reaching the light-sensitive retina (Figure 1). The origin of the word ‘retina’ was linked to the Latin reticulum, referring to an elaborate anatomical network of blood vessels and nerves. At the back part of our eyes, the retina broadly acts as a sensor in a camera; the retina senses the light distinguishing between different wavelengths and intensities and then translates it into an electrical/neural response that is relayed via the optic nerve to our brain.

For the sensory process to function properly, the retina requires various cell types in a complex organized structure formed of two main parts: the inner multilayered neural retina (responsible for sensing and converting light impulses) and the outer monolayer of retinal pigment epithelium, which acts as the supporting tissue. Despite being located away from the brain, the neural retina comprises complex neural circuitry and is part of the central nervous system. The neural retina contains layers of different cells with the outermost layer being formed by the light-sensitive photoreceptors (rods and cones), the primary cells that detect the light in the form of photons. With the difference in their shape and the wavelengths they detect, photoreceptors can be divided into cones (cone-shaped cells tuned for colour vision) and rods (rod-shaped cells adjusted for night vision). After converting the incoming photons into a molecular cascade, phototransduction sends this neuronal response via synapses to interneurons, e.g. bipolar cells, amacrine cells and horizontal cells. The response then flows through retinal ganglion cells that summarize the neuronal response before sending it to our brain via axons (the nerve fibre layer which converges into the optic nerve). Apart from neural cells, the neural retina also comprises supporting cells such as Müller cells, microglia, astroglia, oligodendrocyte Schwann cells, endothelium cells and pericytes (Figure 1). These cells perform housekeeping functions to support the neural tissue, such as exchange of metabolites and protection against injury.

The organization of different cells in the retina also depends on their anatomical location. In the central part of the retina, called ‘macula’, where most of the light is focused allowing us to sense fine details such as colour and facial expressions and to perform particular tasks such as reading a newspaper, various cells are organized differently compared to the non-macular part of the retina. Specifically, in the macula, there is a high density of cone photoreceptors, while the other supporting cells mostly reside in the surrounding area. The outer retinal pigment epithelium underlying the macula, therefore, needs to serve a greater number of photoreceptors, resulting in a different bulkier shape characteristic compared with the flattened shape characteristic of the peripheral areas.

Collaborative work of cell types in the retina to facilitate vision

Key to the intense, constant function of the retina is the collaborative work between photoreceptors inside the neural retina and the retinal pigment epithelium. The photoreceptors have specific structures made by
membrane folding in their outer segment, called discs, which enable the detection and conversion of photons into a biochemical cascade. The discs are loaded with rhodopsin molecules – each one of these formed by a seven transmembrane domain protein (opsin) embedded in the lipid bilayer and a covalently bound retinal molecule. It is estimated that each membrane disc contains approximately $10^5$ rhodopsins, with about $10^8$ rhodopsins per photoreceptor. In its 11-\textit{cis} form, upon absorption of a photon of light, the photoreactive retinal isomerizes to all-\textit{trans} form, thus inducing a conformational change in the opsin.

Normally, without any light stimulus, the photoreceptors have cyclic guanosine monophosphate (cGMP)-gated channels residing in the cell membrane open, allowing sodium and calcium ion flux into the cell. This flux keeps them depolarized and induces glutamate secretion sending information through interneurons by synapsing.

At light onset, the phototransduction cascade begins when rhodopsin molecules with 11-\textit{cis}-retinal absorb a photon, changing into metarhodopsin II with all-\textit{trans}-retinal. The subsequent signal transduction occurs via activation of an associated G protein, transducin, that triggers a cGMP second messenger cascade involving change of cGMP to GMP. The lowered level of cGMP is not enough to keep the channels open, which leads to photoreceptors becoming hyperpolarized and glutamate secretion cessation. This phototransduction mechanism occurs in both types of photoreceptors, but the key difference is that rods have a much higher light sensitivity than cones. As such, low light (scotopic) vision is almost exclusively facilitated by rods, while cones are activated by high-incident light intensity. While the majority of cones are situated in the macula, the rods are found predominantly in the surrounding areas outnumbering the cones, so our peripheral vision is much better at night.

There are four visual opsins in human photoreceptors, each of which slightly alters the chemical environment around the attached retinal molecule, so that its transformation to all-\textit{trans} form and the subsequent cascade are triggered by photon of a different wavelength. Only one opsin is found in rods, which has a peak absorption of blue-green wavelengths – hence, at night, we cannot see red; so, vision becomes monotone. Infants see in the same way, since rod cells are more developed at birth and colour discrimination occurs at only around 3 months of age when cones mature. The other three rhodopsins are found separately in cones, and each of them is most sensitive to one of red, blue or green light. Nearly two-thirds of cones are red sensitive, almost another third are green sensitive and the minority are blue sensitive. The brain interprets various intensity ratios of these wavelengths as the colours we experience and, in fact, can distinguish over 2m colours in this way. Importantly, this process can only be maintained by constantly replenishing the 11-\textit{cis} retinal into the photoreceptors – an essential part for completion of the visual cycle in which the retinal pigment epithelium plays a key role (Figure 2). Following rhodopsin activation, the all-\textit{trans} retinal form is exported out of the discs by ATP-binding cassette protein (ABCR, encoded by the \textit{ABCA4} gene). The retinol dehydrogenase then reduces all-\textit{trans}-retinal to its alcohol form (all-\textit{trans}-retinol), which is transported out of the outer segment to the interphotoreceptor matrix. There, it binds to interstitial
retinal binding protein, and subsequently in the retinal pigment epithelium, where it is transferred and attached to cellular retinol-binding protein. This alcohol form is catalytically converted back into 11-cis-retinal through a chain of multiple enzymes, including LRAT, RPE65 and RDH5. The interstitial retinal-binding protein then transports 11-cis-retinal back to the photoreceptor (Figure 2).

Following the extensive process of phototransduction outlined above and its ensuing oxidative stress damage, the photoreceptors need to regenerate new discs and shed the old ones. The retinal pigment epithelium undertakes the major function of phagocytosis of the spent discs and disassembles them through complex regulated proteolytic processes mediated by lysosomal proteases. An immense amount of material is thus processed over our lifetime, making the retinal pigment epithelial cells some of the most active phagocytic cells in the body. Each retinal pigment epithelial cell will phagocytose hundreds of thousands of discs throughout its lifetime. The remarkable nature of this fact is further evident when considering that without this renewal, the photoreceptors would only be able to function – and hence us to see – for about a week! Both shedding of old discs by photoreceptors and subsequent phagocytosis by the retinal pigment epithelium continuously happen in a diurnal pattern. The level of phagocytosis increases about 2 hours after light onset in rods and after dark in cones. This rhythmic activity may occur due to the suprachiasmatic nucleus in hypothalamus and the retina itself, as even following the experimental removal of the eye, the light–dark pattern of activity of photoreceptors and retinal pigment epithelium continues.

The retinal pigment epithelium is also crucial in other vital processes for preservation of retinal and visual function, including bidirectional transepithelial transport, secretion of growth factors, light absorption and blood–retina barrier function (Figure 3). Each retinal pigment epithelial cell is responsible for the maintenance of about 45 photoreceptors (depending on its topographical location), and unlike other epithelia, the post-mitotic retinal pigment epithelium does not regenerate itself, making it susceptible to cumulative long-term damage. To maintain its normal functions and its high-level metabolism, it requires high amounts of energy and oxygenation. In healthy eyes, these processes...
Dysfunctional retina: genetics and environment

Because there are large numbers of finely tuned processes taking place simultaneously in the retina every day, small defects determined by genetic or environmental factors may lead to multiple dysfunctions, and the unwanted irreversible outcomes can lead to vision impairment and ultimately blindness. Mutations in the visual cycle genes are a group of defects that have been well studied. ABCA4, a 50-exon gene, encodes ABCR that transports all-trans-retinal out of photoreceptor discs and thereby removes the toxic effect this retinoid compound has on the cell. Abnormal function of ABCR due to mutations can lead to failure to remove these compounds and subsequent accumulation in the retinal pigment epithelium following phagocytosis of the discs, resulting in dysfunctional retinal pigment epithelium and photoreceptor degeneration. Owing to the long sequence of the gene, about 900 mutations have so far been discovered, and specific homozygous recessive mutations were causatively linked to Stargardt’s disease. Young people with this disease may present with macular degeneration and begin having a bilateral central visual loss. Other symptoms include visual colour defects, photophobia and poor dark adaptation. Visual acuity can deteriorate early and result in poor prognosis. Unfortunately, there is currently no effective treatment for Stargardt’s disease.

RPE65 gene mutations cause other defects in the visual cycle. This gene encodes a retinal pigment epithelium–specific protein with a molecular mass of 65 kDa, which performs the isomerase function for recovering the 11-cis-retinal in the retinal pigment epithelium. Pathogenic mutations in this gene cause a disease called Leber congenital amaurosis that presents with an early childhood-onset visual loss. The central pathology is due to an ineffective conversion of retinyl ester and accumulation of toxic substances in the retinal pigment epithelium.

Not only the visual cycle defects can cause retinal degeneration, but also other abnormal retinal epithelium functions such as phagocytosis of the outer segments and secretion can lead to irreversible blindness (Figure 4). Cystatin C, the most potent inhibitor of cysteine proteases, is one of the most abundantly expressed proteins by the retinal pigment epithelium and it is predominantly secreted basolaterally at this site. Its precursor form is encoded by the CST3 gene and its N-terminal signal sequence guides cystatin C to the secretory pathway in the retinal pigment epithelium. A homozygous recessive mutation in its signal sequence results in abnormal cystatin C (called variant B) intracellular trafficking and leads to reduced secretion from the cell and some association with...
mitochondria. This mutation in the CST3 gene is one of many risk factors for developing age-related macular degeneration, which is the primary cause of irreversible blindness in the elderly. Major genetic risk factors for increasing the risk of developing age-related macular degeneration have been characterized and extensively reviewed; however, they appear to account for under a half of all cases. Ageing remains the most significant risk factor for developing this degenerative vision condition; hence, current significant research efforts are aimed at characterizing the specific age-related changes pertinent to neural retina and the retinal pigment epithelium.

Environmental factors, including ageing, smoking and oxidative stress, contribute to degeneration of the retina. Epidemiological data evidencing the links of these environmental factors to age-related macular degeneration point most prominently to ageing and smoking, with dysfunctional retinal pigment epithelium being the site of initiating specific pathological mechanisms. These include dysregulation of autophagy, proteostasis, metabolism, inflammation or blood–retina barrier (Figure 4). Consequently, functionally dysregulated retinal pigment epithelium induces photoreceptor degeneration and drusen accumulation (abnormal lipoproteinaceous deposits below its basement membrane, associated with retinal degeneration risk).

**Novel treatments of retinal disease**

Treating the retina and its dysfunction is not straightforward due to a multitude of factors – location and accessibility, complexity and sensitivity of tissue, potential risks and side effects of any intervention, among many others. Over the years, many drugs and procedures were tried aiming to preventing or reduce visual impairment or blindness. Gene therapy has received much attention in recent years in an attempt to reverse the effects of detrimental mutations. Because some of these diseases may progress from mutations in

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**Figure 4.** Retinal pigment epithelium in disease. As retinal pigment epithelial cells are non-regenerative, they are susceptible to cumulative damage from multiple sources as they grow old, leading to dysfunctions in crucial processes that may present as retinal disease. Formation of drusen, extracellular deposits comprising proteins and lipids, is a hallmark of age-related macular degeneration, where retinal pigment epithelium is damaged or dysfunctional. In the exudative form of the disease, the integrity of the blood–retina barrier is compromised, leading to leakage of blood into the retina. Given the intimate relationship with the photoreceptors, dysregulation of the retinal pigment epithelium leads to photoreceptor degeneration.
just one gene, a viable approach is to instruct the relevant cells to express the corrected form of the protein, with the blueprint to do so introduced to the cell using vectors usually derived from the non-virulent part of viruses. In December 2017, the US Food and Drug Administration (FDA) approved the first retinal gene therapy on biallelic RPE65 mutations in patients meeting the criteria of having Leber congenital amaurosis type 2, having a biallelic mutation of this gene and older than 12 months of age. The therapy works by using a subretinally injected adeno-associated virus vector, and clinical trials showed improvement in night blindness and visual functions. While the results of the clinical trials and FDA approval are promising, degeneration of photoreceptors can still occur in treated patients and post-intervention surveillance still needs to be followed up. For some of the other genes discussed here, such as ABCA4, gene therapies are also in clinical trial phases.

Apart from the correction of a single gene via gene therapy, cellular therapy could tackle retinal degeneration. In age-related macular degeneration, complex combinations of both genetic and environmental factors can lead to dysfunctional retinal pigment epithelium, and corrections of just one gene would then not suffice. The overall concept of cell therapy is to replace the dysfunctional or dead retinal pigment epithelial cells with new healthy cells, derived and differentiated from stem cells. The source could be reprogrammed cells from the patients themselves, or induced pluripotent/embryonic stem cells. These are then differentiated to retinal pigment epithelium in vitro and transplanted into the patient’s eye. Like most of the gene therapies, cell therapies are still being tested in initial studies, and preliminary results indicate that this strategy might not reverse the disease completely, but rather delay the progression of the degenerative retina.

Here, we discussed some of the complexities involved in the maintenance of a healthy retina in a typical day, how the different cells collaborate inside the retina to give us the sense of vision, how damage to the retina can lead to serious disease and new techniques allowing us to attempt to fix defects of the retinal function. Our understanding of the inner mechanisms of the retina have gradually transformed from the early descriptions of mysterious 'nets' in the eye to the finely tuned biological machinery we know today. We continue to make progress, uncovering new functions of genes and how cells behave in different scenarios. Clearly, improved understanding of the underlying mechanisms supporting retinal functions will promote new and improved therapies to treat patients with retinal diseases.

Further reading

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