Title: Emerging therapies for retinal degenerations: a cure in sight?

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Introduction

Our sense of vision begins with the conversion of light into electrical signals in the retina of the eye, a process known as phototransduction. Central to phototransduction are the highly specialised photoreceptor cells of the retina: the rods and cones. Rods are needed for night vision, while cones are responsible for colour vision.

Many people are born with impaired vision, and for many others vision deteriorates later in life. Blindness, although not life threatening, is a debilitating disorder which drastically changes an individual’s life in significant and often devastating ways. Retinal degenerations (RDs) resulting in photoreceptor loss are the leading cause of intractable blindness in the developed world. Millions of people worldwide suffer from visual loss due to inherited RDs such as retinitis pigmentosa (RP), or age-related macular degeneration (AMD). RP refers to a group of disorders causing photoreceptor loss typically progressing to involve the central retina. It is the leading cause of inherited retinal blindness in younger patients, with a prevalence of approximately 1:4000\textsuperscript{1}. AMD is characterised by initial degeneration of retinal pigment epithelium (RPE) cells leading to secondary photoreceptor loss. It mainly affects those over 55 years of age, but is much more prevalent\textsuperscript{2}.

Visual impairment due to RDs has high economic and social costs both to the individual and the state. Although the number of individuals affected by RP is relatively small, its early onset causes sufferers to be socially and economically dependent for most of their lives. AMD, on the other hand, is generally a disease of old age but the large numbers of affected individuals together with its effect on independent living and injuries relating to poor vision, such as falls, make this a costly disease both economically and in terms of quality of life.

While the majority of visual impairments, such as cataracts, can be satisfactorily treated by interventions such as surgery, RDs are currently incurable. Therefore research into RD therapies is important for economic and social reasons and also because there is an unmet clinical need.

This essay will review the recent research into novel treatments for RDs. It will also consider some of the current challenges and possible future directions for the development of these emerging therapies.

Emerging therapies

To date, there are few options for the treatment of RD. Supplementation with vitamin A slows disease progression in a subset of RP individuals. Furthermore, it is unlikely that there will ever be a single unifying treatment for all RD patients. This is because different RDs have different underlying pathologies. In addition, there is also much variation in patient age, and in the type, stage and severity of disease.

Emerging treatments for RDs can be broadly categorised into two groups according to the stage of disease: early-stage, when patients still have viable photoreceptors remaining in their retinas; and end-stage, at which time most or all photoreceptors have died.

Gene therapy

Treatment for early stage RDs is aimed at prolonging the life of photoreceptors and improving function. For genetic conditions, one approach is gene therapy. The RD in which gene therapy has been most extensively studied is Leber congenital amaurosis (LCA), a form of incurable early-onset recessive human retinal degeneration caused by a mutation in the retinal pigment epithelium-specific gene RPE65. Human clinical trials reporting prolonged improvement in vision with no major side
effects\textsuperscript{3,4} have not only offered hope for LCA patients, but also increased confidence in gene therapy strategies for RDs in general by demonstrating the safety and efficacy of viral vectors following intraocular administration. RDs that are poorly-characterised genetically would not be amenable to this approach. A major limiting factor for gene therapy is the finite packaging capacity of currently available viral vectors that can safely be used for retinal gene delivery.

**Pharmacological therapy**

Pharmacological agents can be used to treat RDs where the underlying pathophysiology is at least partly understood. For example, oral administration of 9-cis-retinyl acetate in Rpe65-deficient mouse model of LCA resulted in dose-dependent improvements in light sensitivity, as measured by electroretinogram, lasting for six months after therapy\textsuperscript{5}. An advantage of such treatment compared with gene therapy is that drug regimens could be monitored and adjusted to avoid toxicity\textsuperscript{6}. However, as with gene therapy, patients who suffer from RDs with poorly characterised aetiologies would be unable to benefit.

A second pharmacological approach known as ‘neuroprotection’ aims to prevent or slow the degeneration of photoreceptors. This strategy does not compensate for any primary defect, and instead targets common secondary mechanisms underlying photoreceptor cell death. Therefore it can be used in cases where the underlying genetic aetiology is poorly understood. A number of neurotrophic factors that protect against photoreceptor loss have been identified in animal models.

The safe and effective delivery of pharmacological agents to the retina represents a significant challenge for researchers. The blood-retinal barrier prevents most drugs administered topically or systemically from reaching an effective concentration in the retina. Adequate intraocular drug levels with limited systemic toxicity could be achieved by intravitreal injection, but the chronic nature of RDs would necessitate frequent injections leading to risk of retinal detachment. One possible mode of sustained delivery is the use of therapeutic agent-producing encapsulated cells placed in the vitreous cavity. Encapsulated cell technology for the delivery of ciliary neurotrophic factor (CNTF) has been shown to be both safe and effective in different animal models of RP\textsuperscript{7}. Similarly, a phase I safety trial of ten participants with advanced RP using an intravitreal CNTF implant over six months was well tolerated with variable, but positive improvements in visual acuity\textsuperscript{8}. A phase III clinical trial using the CNTF implant is currently being conducted\textsuperscript{9}.

Although gene therapy and pharmacological agents may help to prevent photoreceptor loss in early-stage RDs, they are unlikely to be effective in advanced disease where photoreceptor loss is already established, or when the causative mutations are unknown. Novel therapies are being developed which do not alter disease progression but instead aim to restore photosensitivity in the remaining retinal circuitry. These include retinal cell transplantation, electronic retinal implants, and genetically-encoded light sensors.

**Retinal cell transplant therapy**

RDs are ideal targets for cell replacement therapies. Successful cell transplantation treatment of most nervous system diseases requires replacement neurons to make both afferent and efferent connections with the host, often across long distances and without molecular guidance signals\textsuperscript{10}. Photoreceptors, by contrast, do not have afferent synapses and make only one efferent connection with an adjacent secondary retinal neuron. The retina is also surgically accessible, enabling straightforward delivery of
treatment e.g. by small-gauge vitrectomy, a routine outpatient procedure with low morbidity. In addition, the retina can be visualised through the clear ocular media allowing easy monitoring of the graft site. Uncontrolled proliferation of cells, of particular concern for stem cell therapies, could therefore be promptly identified and readily ablated by non-invasive laser photocoagulation.

There are several potential sources of donor photoreceptors. Whole sheets of foetal neural retina have been transplanted into the subretinal space in rodent models and have survived and differentiated. Improved visually-driven behaviour has been reported, although this could conceivably result from host photoreceptor rescue by donor tissue-derived trophic signals. The foetal tissue approach is limited largely by the physical challenge of transplanting a full-thickness neural retina, including its inner retinal neurons, into the confined subretinal space already containing the host neural retina. The presence of inner retinal components in the donor tissue could interfere with photoreceptors forming connections with the host inner layer. Transplantation of a single sheet of immature photoreceptors might increase the likelihood of synaptic connectivity with the host.

Stem cells, another source of cells for transplantation, are characterised by two fundamental properties: self-renewal and potential for differentiation. In other words, they divide and either give rise to more stem cells or differentiate into more specialised cells. Embryonic stem cells (ES cells), derived from early blastocyst-stage embryos, were previously considered a promising source of stem cells for regenerative medicine because of their ability to mature from an undifferentiated state into each of the more than 220 cell types in the human body. Indeed, human ES cell-derived retinal cells were recently transplanted into a mouse model of LCA and restored some function, demonstrating that human ES cells could, in principle, be used for photoreceptor replacement. However, because ES cells originate from another foetus, patients would require lifelong immunosuppressive therapy. Ethical questions have also been raised about how to obtain the cells. These concerns have fuelled the search for alternative sources of stem cells.

In 2006 it was shown that a cocktail of selected DNA-binding proteins could reprogram stem cells directly from differentiated adult cells. The resultant ‘induced pluripotent’ stem (iPS) cells represent a source of healthy autologous stem cells for transplantation in patients with RDs. Functional human photoreceptor-like cells have been generated from human iPS cells. Since the cells are derived from the patient themselves, there is no risk of immune rejection, thus eliminating the need for chronic immunosuppression after transplantation. Ethical issues associated with ES cells are also circumvented.

iPS cell technology holds great promise for the treatment of RDs. Adult cells from a patient could be reprogrammed into iPS cells and directed towards retinal precursor fate in vitro before transplantation back into the same patient. For patients with inherited RDs, combining cell transplantation with gene therapy of the iPS cells will avoid the possibility of re-implanting cells that carry the original mutation. The hypothetical combination of iPS cells and gene therapy is an exciting future prospect.

Electronic visual prostheses

Electronic prostheses convert photic images into electrical responses and ultimately perception of a visual image. A number of different approaches and technologies are being developed.

One strategy is to bypass the eye completely and directly stimulate the visual cortex. Brindley and Lewin in 1968 were among the first to pioneer this approach. Surface stimulation of the occipital pole of the right cerebral hemisphere by "an array of radio receivers" caused a blind patient to "experience sensations of light ('phosphenes') in the left half of the visual field". The concept of a cortical visual
prosthesis was further boosted by demonstration that more modest levels of electrical currents passed directly into the cortex can produce sensory percepts\textsuperscript{20}. A clinical trial on intracortical visual prostheses is being planned by a group from the Illinois Institute of Technology\textsuperscript{21,22}. Most research is being directed towards the development of intraocular retinal implants. The precedent for a retinal prosthesis was set by the success of the cochlear implant for treating deafness, which demonstrated that the auditory pathway could use artificial sensory input from an electronic device\textsuperscript{23}.

Retinal implants convert light images into electronic signals, which are sent to secondary retinal neurons (ganglion cells, bipolar cells, etc.) that in turn transmit them to the visual cortex via the optic nerve and radiation. Therefore the success of any retinal prosthesis relies on the presence of functional secondary neurons in the retina. Morphometric studies have demonstrated viable secondary neurons in patients with severe or moderate RP\textsuperscript{24}. Matsuo and Morimoto used optical coherence tomography to assess the presence of viable retinal neurons in RP patients, correlating visual acuity with retinal thickness. They postulated that this technique “may be used as a clinical test to assess retinal prostheses in the future”\textsuperscript{25}.

Although morphometric studies show significant numbers of secondary retinal cells in RDs, many of these cells are histologically abnormal in advanced disease\textsuperscript{26}. Central connecting neurons may also degenerate through lack of visual input. Such abnormalities could conceivably affect retinal and central cell function, thereby reducing the chances of success of retinal implants. However, stimulation of the retinal surface in blind subjects with end-stage RP or AMD elicited the perception of discrete spots of light\textsuperscript{27}. This suggests that sufficient integrity of the visual pathway remains in advanced RD, even after years of little visual input, for at least some rudimentary signals to be transmitted.

Electronic implants are placed directly on the retina using a subretinal or epiretinal approach. Subretinal implants are placed in the subretinal space between the pigment epithelial cells and dying or dead photoreceptors. Zrenner et al. recently implanted a subretinal prosthesis containing an array of 1500 active micro-photodiodes (‘chip’) into three blind human subjects and restored visual percepts such that they could localise bright objects on a dark table and recognise typical shapes such as a banana. One patient was even able to read large print\textsuperscript{28}.

In the epiretinal approach, implants are tacked onto the vitreal (front) surface of the retina. The Argus II retinal prosthesis system (Second Sight Medical Products, Sylmar, California, USA), is an epiretinal implant system consisting of a video camera mounted on glasses, a video processing unit (VPU) and a 60-electrode microelectrode array that stimulates the inner retina directly. In a recent prospective, internally controlled multicentre trial of the Argus II system, 28 blind subjects with light perception vision consistently identified letters and words, indicating reproducible spatial resolution\textsuperscript{29}. This year, the Argus II system received US market approval from the Food and Drug Administration (FDA) to treat individuals with late-stage RP\textsuperscript{30}, making it the only FDA-approved, long-term therapy for advanced RP.

Although these results provide hope that electronic prosthetic devices will restore vision in the blind, not all patients will benefit to the same extent and many will only experience small improvements. Current tests for visual function such as visual acuity and visual field are insufficient for assessing very poor vision and the small changes that occur with these early implants. Standardised methods need to be developed to assess visual performance with electronic prostheses that can detect small
improvements in patients with very low vision. They should also include items which relate to better real-world functioning and quality of life\textsuperscript{31}.

**Optical photoswitches**

Light-activated ion channels represent a novel approach, besides retinal cell transplantation and electronic devices, of replacing lost photoreceptor input in the visual pathway. This ‘optogenetics’ strategy involves genetically encoding light-sensors into retinal cell membranes, thereby converting them into artificial photoreceptors. Like electronic prostheses, optogenetics aims to offer functional vision using only the non-photoreceptor secondary neurons of the retina. The main advantage of this approach compared with electronic prostheses is that light sensors could be targeted to specific retinal cell types, enabling artificial retinal activity to more closely resemble normal retinal activity.

Optogenetic approaches are currently at the phase of pre-clinical trials. Blind transgenic rodents expressing the bacterial light-gated membrane ion channel Channelrhodopsin-2 in a subset of ganglion cells regained retinal photosensitivity and showed some restoration of basic vision driven behaviour\textsuperscript{32,33}. Similarly, Busskamp reactivated light-insensitive cones and enabled visually guided behaviours in two mouse models of RP by virally-mediated gene transfer expression of bacterial halorhodopsin\textsuperscript{34}.

**Conclusion**

In summary, RDs are an important cause of blindness in the developed world. To date, there are no proven therapies to stop disease progression or restore vision. However, novel therapeutic approaches are being developed for patients with either early-stage disease, where there are still surviving photoreceptors, or late-stage disease, where few photoreceptors remain.

Translational clinical research initiatives offer hope to RD patients but we are still some way off a cure. Although the molecular pathophysiology of these conditions is gradually being revealed, accurate and comprehensive characterisation of this heterogeneous group of disorders is a major challenge. While elucidation of all the underlying pathologies should remain a long-term target for researchers, identification of the pathophysiological processes common to all the photoreceptor degenerations may represent a more realistic immediate goal to facilitate the development of a more generic treatment for the different types of RDs.


