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Original Article

Exploring Gene Therapy as a Cure for Age-Related Macular Degeneration

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Abstract - *Age-related Macular Degeneration (AMD) is a complex disorder and a major cause of blindness in elderly people across the globe. This condition originates at the retina but spreads to the inner layers of the eye. In AMD, the macular lining of the eye, which is primarily involved in vision, becomes damaged. Consequently, straight lines appearing wavy and distorted are observed as an early symptom of AMD. Multiple factors, including genetic history, lifestyle, and reactive oxygen stress cause the inception of AMD. Several advanced therapies are in clinical trials for the treatment of this life-changing disorder, but none of them promise a perfect cure. Gene Therapy aims to deliver the correct copy of the RPE65 gene (which is responsible for the condition) to the retina and help in the production of the proper RPE65 protein to maintain vision. An advantage of this method is its potential to reduce the number of continuous injections, unlike other therapies under trial.*

Keywords - *Age-related Macular Degeneration (AMD), RPE65 gene, Retina, Gene Therapy.*

1. Introduction

Age-related macular degeneration (AMD) is the chronic and progressive degeneration of photoreceptors, the underlying retinal pigment epithelium (RPE), Bruch's membrane, and potentially the capillaries in the macula. Predominantly seen in the later stages of one's lifespan, it is a major driving force of vision loss in middle-aged people. Approximately one-tenth of people in the United States are affected by this condition [1]. Age-related macular degeneration is categorized in three stages – Early, Intermediate, and Late, the latter causing irreversible blindness. Late AMD can be further subdivided into two subcategories – Dry and Wet. Dry AMD is caused by the accumulation of waste material called drusen and is associated with the disordered arrangement of pigments in the retina, which is essential for clear vision [2]. Wet or Neovascular AMD is an advanced form and occurs when age-related conditions worsen, disrupting existing retinal tissue, causing irreversible damage and vision loss. This defect has a range of causes, including both physiological and hereditary factors. AMD is incurable and irreversible in its later stages, because of which the development of a therapy for the condition is critical. Currently, most of the treatments available for AMD are administered to those patients with the advanced, neovascular form. However, no therapeutic agent can effectively prevent the irreversible apoptosis and loss of RPE and photoreceptor cells. This is where gene therapy comes in. In gene therapy, a single dose can cure a lifelong illness by helping in the production of endogenous genes, which is otherwise not possible due to the unwanted mutations in the RPE65 gene. Although gene therapy could involve detrimental immune reactions and other side effects, in addition to its high cost, limited access and ethical concerns, it serves as the most promising method to aid the fight against genetic diseases such as AMD. Thus, gene therapy has been chosen for this paper to target the condition at the genetic level and control its expression, saving the eyesight of humanity [3].

The novelty of this paper is evident through both the lack of public awareness regarding AMD as well as the lack of funding for gene therapy, because of which such research is termed a "waste" and not explored, especially since AMD is not life-threatening but affects an individual's lifestyle. While a plethora of research exists for the treatment of wet AMD, due to its causative agent being an abundance of growth factors (which can be controlled), treatment via targeting a gene in the entire human genome to cure dry AMD is not a focus in current existing manuscripts.

2. General Pathology

2.1. Accumulation of Drusen

When an individual has developed AMD, a cascade of vision-impairing events takes place in the retinal layer. The primary change taking place in the macula is a result of the aging of the Retinal Pigment Epithelium (RPE), which is a specialized cells that maintain a balance of photoreceptors and excrete metabolic waste products through the Bruch's Membrane located between the RPE and the choroid [4]. With age or damage, these cells gradually accumulate increased concentrations of lipofuscin, a byproduct of incomplete metabolism. Lipofuscin accumulates between the RPE layer and the Bruch's membrane, creating drusen that damage these layers and impair vision. This drusen

serves as a biomarker for AMD, and the concentration of drusen indicates the stage of progression of the defect.

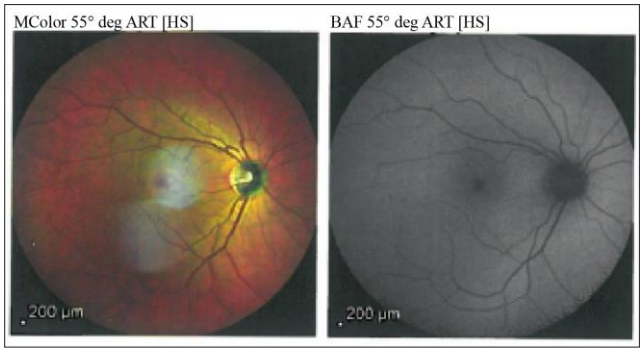

Fig. 1 Formation of drusen

2.2. Formation of Reactive Oxygen Species (ROS)

The retinal layer of the eye has a significant oxygen demand, and the macula faces constant stress due to prolonged photon exposure and massive oxygen utilization [5]. This use of oxygen generates reactive oxygen species (ROS) such as hydroxyl ions that are usually involved in regular metabolism for energy. The RPE cells contain vast amounts of antioxidants, which maintain homeostasis of harmful radicals, including ROS, in the retina and prevent retinal damage from the ROS. In AMD, due to atrophy of RPE, the antioxidant content falls short of the required amount to counterbalance the harmful ROS, resulting in a disruption to homeostasis and creating oxidative stress. This impairs the proper functioning of the existing RPE cells. Furthermore, the Bruch's Membrane serves the function of transporting oxygen from the choriocapillaris to the RPE cells. However, due to drusen accumulation, oxygen cannot reach the RPE, worsening the conditions of oxidative stress. This results in further death of RPE cells.

2.3. Vascular Endothelial Growth Factor (VEGF)

Wet AMD or neovascular AMD is caused by the unregulated expression of a protein known as Vascular Endothelial Growth Factor (VEGF), causing an abnormal growth of the choriocapillaris. This abnormal choroidal neovascularization in an eye that cannot accommodate more vessels results in leakage of blood or serum, causing central vision loss [6].

2.4. Complement System

The complement system is involved in the innate immune response and comprises several proteins and their pathways, serving the purpose of defence against foreign substances [7]. The complement system has three different pathways - Classic, Alternative, and Lectin, which all produce the same product of C3 convertase. This protein undergoes a series of steps to form a Membrane Attack Complex (MAC) that penetrates the cell membrane and induces cell lysis [8]. In AMD, due to atrophy of RPE, an inflammatory response is initiated in the subretinal area. This inflammation results in the formation of C3 convertase, creating the MAC and causing greater drusen deposition. The levels of some of the inflammatory proteins involved in this response, such as C-reactive protein (CRP), serve as an important biomarker for AMD. Genetic factors can also affect the complement system and become a direct cause of AMD. A mutation in the complement factor H (CFH) gene that usually encodes an inhibitor molecule preventing the formation of MAC loses its specificity and cannot perform its function, resulting in greater apoptosis in the cells of the retina [9].

3. Screening

After the subretinal changes mentioned in Section II manifest in a patient and they experience compromised vision, an ophthalmologist will screen the eye to determine the presence and level of AMD and decide on the plan for treatment. This screening is done through various methods.

3.1. Optical Coherence Tomography (OCT)

This is the most widely chosen tool for screening due to its non-invasive nature. In this process, retinal cross-section images are obtained from light waves. By measuring retinal thickness, the atrophy of RPE and buildup of drusen can easily be identified. In case of neovascular AMD, the fluid from subretinal hemorrhage can be detected [10].

3.2. Fundus Autofluorescence (FAF)

Based on the fluorescent principles of lipofuscin, blue light is directed into the eye (Wavelength = 470 nm), which interacts with the bisretinoids, a main component of Lipofuscin, and re-emits green-yellow light of 600nm. By measuring the emission of 600 nm light, a density map of lipofuscin can be created that tracks RPE atrophy [11].

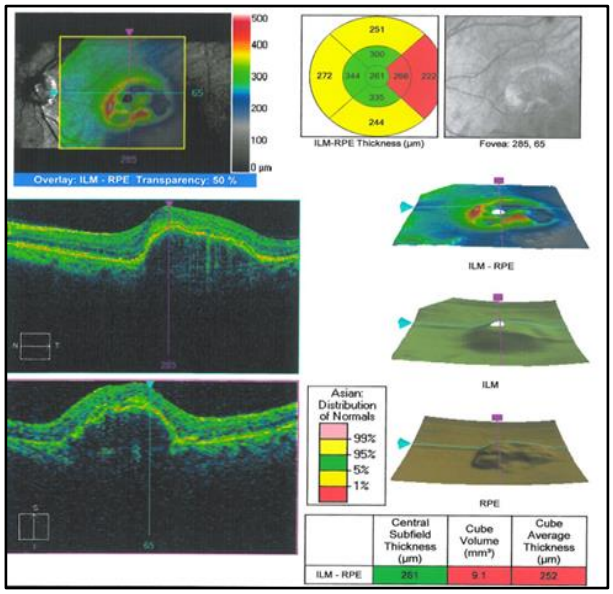

Fig. 2 Layer-by-layer Optical Coherence Tomography Scan of AMD retina

3.3. Indocyanine Green Angiography (ICGA)

ICGA is based on the principle of choroidal circulation, providing nutrients to the inner layers of the eye. Indocyanine is injected into the veins and circulates to the eye, creating an angiogram of the vessels of the eye. This can be used to detect VEGF levels causing choroidal neovascularization. However, this method is not preferred because the procedure is invasive, the pigment is expensive, and the results are inconsistent. [12].

3.4. Amsler's Grid

This is the most convenient method of screening and can be done even at home without an ophthalmologist [13]. Amsler's grid contains a dot and a simple square pattern, as shown in the image. Distortions or black spots in the grid can be early signs of AMD as they indicate the buildup of drusen in the eye. [14]

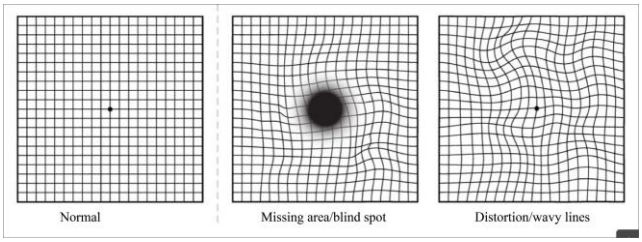

Fig. 3 Amsler's grid

4. Therapies

Currently, several therapies are being experimented with for the treatment of AMD.

4.1. Laser Photocoagulation

As mentioned earlier, overexpression of VEGF results in choroidal neovascularization and fluid leaking [15] that damages the macula. When anti-VEGF medications are proven to be ineffective, laser photocoagulation may be conducted. In this process, a lens concentrates a beam of light onto the retina, which seals off abnormal blood vessels and prevents further leaking. While this has proven to be effective, it only slows down vision loss and is not a permanent solution.

4.2. Targeting the Complement System

During AMD, the atrophy of RPE initiates the complement system, which creates Membrane Attack Complexes to cause cell lysis. This results in further cell death of RPE. However, medications such as Pegcetacoplan inhibit the formation of this MAC by curbing the formation of C3 convertase, the initial molecule in the complement cascade. This cannot cure AMD, but it can prevent the complement system of the body from worsening macular conditions [8].

4.3. Injecting Monoclonal Antibodies

In this type of treatment, several antibodies that are identical clones are injected into the body. These serve the purpose of marking antigens to be destroyed by the immune system. For AMD, an antibody, Vabysmo, exists that marks VEGF proteins to be targeted by the immune system. This prevents further formation of blood vessels, slowing down the inception of AMD. [16]

4.4. Gene Therapy

The most promising treatment for arresting the condition is gene therapy, which has been specifically chosen for this paper. Gene therapy has shown success in all medical fields and has the potential to change human evolution by editing the genome. In gene therapy, a vector encoding the gene of preference is injected into the body and finds its way to the target cell, where it is incorporated into the genome using restriction enzymes. As aforementioned, AMD can be due to both environmental factors and genetic factors. The main genetic cause is the mutation in the RPE65 gene [17]. The RPE65 gene produces a protein which acts as an isomerase enzyme, catalyzing the transformation of one geometric isomer, 11-trans retinol, to another, 11-cis retinol. This triggers a cascade of reactions that are ultimately involved in the process of vision. The mutation of RPE65 prevents this isomerization and hence can cause irreversible blindness. In gene therapy, a plasmid with the correct RPE65 copy will insert itself into the genome, allowing proper vision.

5. Gene Therapy

In the process of gene therapy, the most chosen research messengers are Adeno-Associated Viruses (AAV). AAVs are chosen as vectors not only in gene therapy but also in many other advanced therapies because they are non-pathogenic, and their replication can be easily controlled. AAVs belong to the parvovirus family group, and their genome consists of a single-stranded DNA molecule of around 4.8 kilobases. AAVs depend on co-infection with other viruses, such as adenovirus, which assist in replication. This single-stranded genome consists of three different genes known as Rep, involved in replication inside a host, Cap, involved in capsid formation of the virus, and the assembly (AAP), which increases the efficiency of transfection. These coding sequences are flanked by ITRs (inverted terminal repeats) that are needed for genome replication and packaging, serving as the origin of replication in the recombinant plasmid. The Rep gene encodes four proteins (Rep78, Rep68, Rep52, and Rep40), which are vital for viral genome replication, while the Cap gene expression gives rise to the viral capsid proteins (VP; VP1/VP2/VP3) that form an outer capsid shell to protect the viral genome. It is also actively involved in cell binding and endocytosis. [18]

When a cascade of subretinal changes takes place in the RPE layer of the eye, the inception of AMD takes place. A mutation in the Retinal Pigment Epithelium *65 kDa* segment is the root genetic cause for AMD. Gene therapy aims to target the faulty RPE65 gene as a cure, curbing the condition and restoring the gift of vision.

The RPE65 gene is accommodated in the viral genome through restriction enzymes and is delivered inside the human body using an inactive AAV virus [8]. The transduced cells then start producing the correct RPE65 protein. This allows the protein to successfully catalyze the cis-trans isomerism conversion and hence restores the conditions of normal vision. As a result, genetically caused AMD can be cured by gene therapy, with the downside of the high costs required for the process. The recombinant protein production starts by choosing an appropriate mammalian cell line for transfection with the AAV virus carrying RPE65, the gene of interest. Due to ease of transfection and higher productivity, Human Embryonic Kidney (HEK) 293 remains the first choice when it comes

to the production of any recombinant proteins for advanced therapies. The cells are transfected with the virus containing the gene of interest, Rep, Cap, and a helper gene that allows AAV to replicate. As the cell cycle continues, it amplifies the gene of interest. This is followed by cell lysis, breaking open the cell membrane and releasing the encapsulated viruses with the RPE65 genes. [19]. Purification is conducted to ensure the removal of empty capsids from the intact virus, ensuring the quality of the therapy. The encapsulated viruses are quantified to find out the virus titer produced, which can help in estimating the dose for patients. Research has shown that AAVs belonging to the second serotype (Strain) have successfully been proven to be safe and effective, and hence are being used in clinical trials to research gene therapy.

6. Conclusion

AMD is indeed a complex condition. Unhealthy activities like smoking or having a diet rich in saturated fats initiate the condition, which can gradually worsen with time. Furthermore, AMD is also inherited through generations. The development of a proper treatment is critical, not only to prevent the defect but also to cure the affected patients. Various diagnoses are successfully in place to understand the stage of AMD and changes happening inside the eye upon its onset. The major concern now remains the therapies to cure this disorder. Evaluating the current courses of treatment, gene therapy emerges as a promising option. AAV vectors have proven to be a stable, innovative mediator that can be used in the treatment. In the last few decades, gene therapy has evolved from merely a conceptual phase to clinical trials for over one thousand four hundred medical disorders. The overall result of the trials and advancement of this field over time, if successful, can completely re-establish modern medicine, eradicate diseases currently considered 'incurable', and even allow humanity to influence evolution.

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