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Gene Editing: New Opportunities for the Treatment of Age-related Macular Degeneration

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Abstract: *Currently, the population aging is becoming increasingly severe, and the incidence of age-related macular degeneration (AMD) has also risen, emerging as an increasingly significant public health concern. This disease affects individuals over 55 years old and poses a threat to the high-resolution central vision necessary for essential human activities. There have been numerous hypotheses and investigations regarding the etiology of AMD. Research indicates that the onset of AMD is associated with factors such as oxidative stress, inflammatory responses, cell necrosis, and drusen. At present, the majority of treatments for neovascular age-related macular degeneration mainly involve injecting anti-VEGF antibodies to inhibit angiogenesis and delay the progression to blindness. In recent years, studies on the correlation between the complement pathway and AMD have been quite prevalent. Moreover, various genetic eye diseases have been treated using gene editing approaches. Hence, this review focuses on the pathogenesis of AMD and its related complement pathway, and provides an outlook on the application of gene editing therapy for AMD.*

Keywords: *Age-related macular degeneration, Gene editing, CRISPR-Cas9, Anti-vascular endothelial growth factor (VEGF) drugs, complement*

1. Introduction

Age-related macular degeneration (AMD) is a common eye disease and one of the leading causes of blindness in the elderly. The etiology of age-related macular degeneration (AMD) is understood to be a multifaceted interplay of genetic and environmental determinants. In its initial phases, disease advancement is theorized to be linked with oxidative injury to the retinal pigment epithelium (RPE), precipitating the demise of RPE cells and the progressive aggregation of inflammatory mediators, subsequently leading to localized pigmentation alterations and the development of drusen[1]. Recent studies have revealed that abnormal regulation of the complement system is also closely related to the onset of AMD.

2. The pathogenesis of age-related macular degeneration

The pathogenesis of AMD is not fully understood, and existing research suggests that the disease stems from a combination of genetic and environmental factors. The following five factors are the ones that have been discussed more frequently.

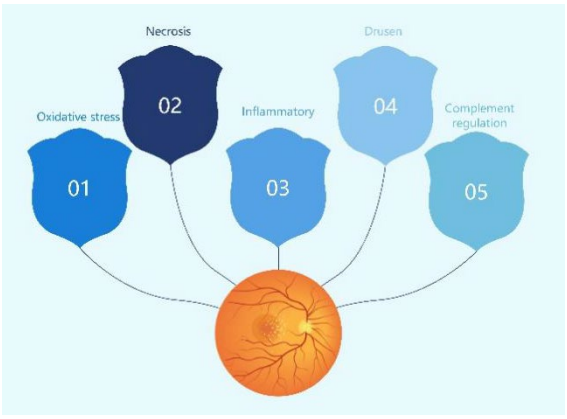

Figure 1: The pathogenesis of age-related macular degeneration.

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2.1. Oxidative stress

The human retina, characterized by its substantial oxygen consumption, encounters an intricate interplay of factors contributing to heightened oxidative stress. Factors including intense oxygen metabolism, continual exposure to light, elevated concentrations of polyunsaturated fatty acids, and the presence of photosensitizers collectively foster an environment conducive to heightened reactive oxygen species (ROS) production within the retinal milieu. Three primary cellular sources of ROS production are identified: macrophages, the mitochondrial respiratory chain, and lipid peroxidation affecting mitochondrial polyunsaturated membranes. Central to this paradigm is the recognition of oxidative stress and associated injury as paramount triggers for the degeneration of the retinal pigment epithelium (RPE), with mitochondrial locales serving as principal sites for excessive ROS generation. The potent reactivity of ROS compounds poses the potential to disrupt the normal functions of intracellular biomolecules[2]. The macular region of individuals afflicted with age-related macular degeneration (AMD) reveals the presence of various lipid peroxidation byproducts, encompassing advanced glycation-end products, carboxyethylpyrrole, malondialdehyde, and 4-hydroxynonenal[3]. Concurrently, disruption in the coordinated regulation of protein synthesis, folding, and degradation is evident, accompanied by the accumulation of autofluorescent lipofuscin, cytoplasmic aggregates, and riboflavin within the macular environment. Photoreceptor cells (PR) within the retina, perpetually exposed to light and oxygen, exhibit pronounced susceptibility to oxidative damage. Simultaneously, degeneration within the RPE cell layer, attributed to oxidative stress and other stressors, culminates in the secondary demise of PR cells. Typically, oxidative damage is counteracted through the actions of antioxidants and intracellular repair mechanisms, or by means of phagocytosis by macrophages. However, as oxidative damage escalates, the body's antioxidant capacity diminishes, and the repair system becomes progressively less effective in its response. The accrual of damage at this juncture precipitates retinal dysfunction and ensuing impairment of vision. Notably, AMD patients display evidence of a protein degradation defect within the RPE, characterized by elevated levels of ubiquitin (Ub) and p62/SQSTM1, indicating dysfunctionality in the Ub proteasome and lysosome/autophagy pathways, respectively[4].

2.2. Necrosis

Stress has been established as an instigator of cell death within the retinal pigment epithelium (RPE) both in controlled in vitro settings and in vivo contexts. Recent investigations have unveiled necrosis as the predominant mechanism governing RPE cell demise in response to oxidative stress, an occurrence known to incite inflammatory and immune responses. Within this framework, high mobility group box 1 (HMGB1) emerges as the principal damage-associated molecular pattern (DAMP) molecule, undergoing passive release from the nucleus and subsequent secretion into the extracellular matrix during necrotic events[5]. HMGB1's pivotal role is underscored by its capacity to induce the expression of the inflammatory gene tumor necrosis factor (TNF) in both RPE cells and macrophages, with the latter secretion originating from necrotic RPE cells. Experimental evidence substantiates this phenomenon, as TNF expression levels declined upon the introduction of HMGB1 antibodies into conditioned media, affirming HMGB1's central involvement in provoking the expression of inflammatory genes within necrotic RPE cells[6]. Multiple lines of support for the occurrence of RPE cell necrosis are presented: (1) Morphological evidence elucidates necrotic features such as cellular swelling, roundness, and vacuolation, observed not only in in vitro models of oxidative stress induced RPE cell death but also in mouse models of age-related macular degeneration (AMD), with a particular prevalence in geographic atrophy (GA) cases among AMD patients[7]. (2) Molecular evidence further consolidates this notion, highlighting the activation and aggregation of receptor-interacting protein kinase 3 (RIPK3), the release of HMGB1 from the nucleus and its subsequent secretion, and the successful rescue of oxidative stress-induced RPE cell death through the utilization of RIPK1 inhibitors, such as necrotitin, and RIPK3 silencing[7-8]. These collective findings provide compelling support for necroptosis as the primary modality of cell death in RPE cells in response to oxidative stress.

2.3. Inflammatory

In the pathological context of age-related macular degeneration (AMD), an abundance of reactive oxygen species (ROS) and oxidized lipoproteins contributes to protein misfolding, aggregation, and the sustained activation of the immune response. Concurrently, chronic inflammation in AMD may arise from the necrosis of retinal pigment epithelium (RPE) cells. The retina, as an immune-privileged tissue, safeguards itself against both external and internal threats through the blood-retinal barrier and an immunosuppressive microenvironment. Additionally, the retina possesses its own defensive mechanisms,

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comprising microglia, T cell activation[9], and the complement system. These components, combined with immune privilege, collaboratively maintain retinal homeostasis. Over the course of aging, retinal cells are exposed to progressively heightened levels of oxidative stress, which can impair their neuronal function and immunomodulatory capacities. Under normal physiological circumstances, neurons exert regulatory control over retinal microglia and the complement system by producing various inhibitory molecules, both membrane-bound and soluble. In instances where circulating immune cells infiltrate the retina, these molecules can induce immune cell apoptosis and foster their transformation into immunosuppressive cells[10]. During the aging process, substantial morphological and functional changes occur in RPE cells. Their numbers diminish, size increases, and a notable proportion becomes binucleated or multinucleated. Typically, healthy RPE cells possess the capability to modulate the phenotype and function of macrophages, enhancing their capacity for debris removal. In the aging retina, the expression of complement regulators is generally downregulated, while complement protein expression is elevated. This complex interplay can be attributed to multiple mechanisms[10]. Additionally, inflammatory cytokines released from subretinal macrophages may upregulate complement protein expression and downregulate complement regulator expression in RPE cells[11].

2.4. Pathological deposits-Drusen

Drusen, pathological deposits located between the retinal pigment epithelium (RPE) and the Bruch membrane, represent a significant risk factor in the pathogenesis of age-related macular degeneration (AMD). In the early stages of AMD, the primary manifestations are observed within the RPE and Bruch's membrane. Notably, the RPE progressively accumulates lipofuscin, leading to the presence of partially digested photoreceptors within its cytoplasm[12]. Unique to retinal lipofuscin is its lipid-rich composition with minimal protein content, exhibiting characteristics akin to traditional particles, including resistance to enzymatic digestion, which can induce lysosomal swelling, subsequently culminating in lysosomal rupture or functional impairment. Furthermore, it possesses the capacity to incite NLRP3 inflammation, a hallmark of compromised lysosomal integrity. Analogous to conventional lipofuscin, it serves as a naturally occurring fluorochrome, regarded as a form of toxic waste, and serves as a biomarker of aging[13]. Drusen composition encompasses acute phase proteins (e.g., CRP, vitrelectin, α-anticoagulant trypsin, amyloid P component, and fibrinogen), components of the complement pathway (C3, C5, MAC complex), inhibitors (clusters), apolipoprotein B and E, mucopolysaccharides, lipids, mannose, and sialic acid. Diminished levels of the endoplasmic reticulum chaperone, ERp29, have been implicated in the accumulation of misfolded proteins, thereby disrupting intracellular motility. This disruption ultimately fosters the aggregation of lipofuscin and the formation of drusen, ultimately contributing to damage to the retinal pigment epithelium (RPE).

In advanced stages of age-related macular degeneration (AMD), the emergence of choroidal neovascularization, a prominent characteristic of wet AMD, stands as the primary cause of vision impairment in affected individuals. The choroidal vasculature, distinctive in its structural and functional attributes relative to other vascular networks, exhibits heightened blood flow and oxygen content, albeit with a pericyte coverage of capillaries merely one-eighth that of other vasculatures. Such structural vulnerabilities render the choroidal vasculature susceptible to pressure-induced variations, fostering the development of neovascularization. Most instances of neovascularization culminate in the disruption of Bruch's membrane within the retina, precipitating focal retinal detachment and subsequent vision loss[14].

2.5. Complement regulation

Through genetic and epidemiological studies, the onset of AMD is associated with more than 35 kinds of genetic variations, and most of them have significant correlations with the complement system. Meanwhile, existing studies have shown that the complement activation products in AMD patients are elevated. Among them, the complement activation levels in patients with intermediate AMD and late dry AMD are significantly different from those in the control group and the early AMD group. There is also an increase in complement activation products in patients with wet AMD, but the increment is smaller than that in the above two types of patients. This is more prominent in patients with defective CFH genes[15]. The dysregulation of the complement system is also associated with the other pathogenic mechanisms discussed above. When the cultured cells are exposed to H2O2 or other sources of oxidative stress, changes related to the complement system can be observed, such as the increase in C3 levels, the decrease in CFH gene expression, and the reduction of FH both inside and outside the cells[16]. Complement activation will recruit and gather immune cells in nearby tissues, triggering an inflammatory response. C3a and C5a can cause mast cells to degranulate and release proteolytic substances, leading to

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the degradation of extracellular matrix[17]. FH has a protective effect on extracellular matrix to prevent excessive deposition of C3b. Therefore, when the FH gene is defective, the related population is more prone to AMD than the FH-normal population.

3. Drugs for treating AMD

Currently, the first-line treatment for AMD is intravitreal injection of anti-vascular endothelial growth factor (VEGF) drugs. Four drugs are already in use -- pegatinib sodium, branibizuma, bevacizumab and aflibercept. At present, there are also a large number of clinical studies on drugs targeting complement regulation, and substantial results have been achieved.

The C3-targeted drug Compstatin (POT-4, AL-78898A), a 13-residue cyclic peptide, selectively binds to C3b and C3c to inhibit complement activation. It has shown some clinical efficacy in its Phase I trial (NCT00473928), But two phase 2 trials (NCT01157065, NCT01603043) failed to replicate the success of phase 1. Given the early success Compstatin has already had, it still has value. New trials with improved design and dosing should be conducted to reassess feasibility. Derivatives of the Compstatin family (e.g., AMY-101, Amyndas, PharmPharmticals) are currently the only low molecular C3 inhibitors through clinical development for the treatment of several indications[18]. For example, APL-2/ Pegcetacoplan is a synthetic cyclic peptide, Which binds to polyethylene glycol polymer 35 and specifically binds to C3 and C3b, blocking the activation of all three complement types. NCT03465709, NCT02503332 and other clinical trials can demonstrate its effectiveness. On February 17, 2023, the complement C3 cyclopeptide inhibitor APL-2/Pegcetacoplan was approved for the treatment of dry AMD, which marked a major breakthrough in the field of complement drugs in common diseases.

Eculizumab blocks C5a and C5b, thereby inhibiting the downstream pathway that causes AMD. However, this inhibition has not contributed to the therapeutic effect so far.

LFG316 is a whole-person IgG1 that targets C5 and inhibits CP or AP. In its early experiments, results showed that LFG316 could bind to C5 and prevent it from cutting. However, LFG316 did not show any success in a 12-month phase 2 trial announced at the Vascular, ko Formation, Exudation and Denaturation conference in Miami, USA on 6 February 2016.

The anti-complement factor D Lampalizumab(FCFD4514S), by binding to the C-terminal portion of Fd, effectively and selectively blocks AP activation. Anti-b factor Ionis-Fb-LRx(ISIS 696844)Ionis-Fb-LRx affects AP by directly reducing the production of Fb. It was tested in a Phase I trial (ACTRN12616000335493) in 2017 with a sample size of 30 participants to evaluate the safety and tolerability of subcutaneous injections at two different doses (10 mg and 20 mg). The trial has since been withdrawn due to a change in commercial objectives. Other antibodies that block the AP pathway at B-factor levels are still in animal trials, and there are no clinical trials available on ClinicalTrials.gov.

But these treatments require frequent and sustained intraocular injections, as well as good adherence. Frequent injections can also lead to other adverse events such as endophthalmitis. At the same time, studies have shown that long-term use of drugs targeting a single target of VEGF gradually reduces the efficacy, and one of the clinical countermeasures is to start to develop drugs that work on multiple targets, including VEGF and complement pathways. IBI302(Innovent.IBI302) is a novel bispecific tricking receptor fusion protein, which can simultaneously inhibit the cascade reaction of vascular endothelial growth factor and complement. Its affinity profile and pharmacokinetics have been evaluated in vitro and in rhesus monkeys[19]. Relevant clinical trials have been conducted, but not yet available on clinic.gov. The results are not yet available online.NCT04370379

Avacincaptad pegol/ARC1905 is a selective C5 inhibitor. Complementing anti-VEGF therapy with complement inhibitors, such as Zimura, is considered to have the potential to further enhance the efficacy of anti-VEGF monotherapy for wet AMD. The Phase IIb trial (NCT02686658) was designed to evaluate the safety and efficacy of Zimura monotherapy when administered intravitreal in GA patients. Because the C5 inhibitor 115 theoretically retains C3 activity, it may offer an additional safety advantage[20].

The Phase 2 study (NCT03362190), with an estimated enrollment of 60 patients, was completed on November 15, 2018. Its objective is to determine the safety and durability of Zimura in Combination With Lucentis®0.5 mg in wet AMD NCT03362190. In August 2023, the complement C5 protein inhibitor Izervay/avacincaptad pegol received FDA approval for clinical use.

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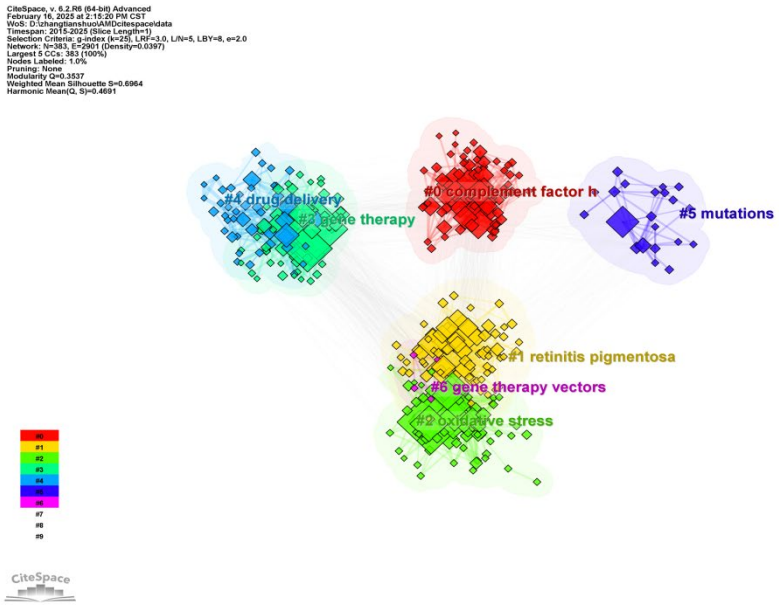

Figure 2: The keywords of relevant literature from 2015 to 2025 were analyzed using CiteSpace (search terms: age-related macular degeneration AND gene therapy).

Top 35 Keywords with the Strongest Citation Bursts

Keywords	Year	Strength	Begin	End	2015 - 2025
ocular neovascularization	2015	4.57	2015	2017	
polymorphism	2015	3.77	2015	2016	
susceptibility	2015	3.75	2015	2016	
lebers congenital amaurosis	2015	3.16	2015	2018	
vascular endothelial growth factor	2015	3.11	2015	2017	
epithelium derived factor	2015	3.1	2015	2019	
age related macular degeneration	2015	2.99	2015	2017	
intravitreal ranibizumab	2015	2.89	2015	2017	
retinal degeneration	2015	2.69	2015	2016	
neovascular age-related macular degeneration	2015	2.57	2015	2017	
model	2015	4.62	2016	2020	
leber congenital amaurosis	2015	2.64	2016	2019	
growth factor treatment	2017	3.24	2017	2018	
pigment epithelial cells	2015	4.33	2018	2021	
rcs rats	2018	2.73	2018	2019	
diabetic macular edema	2019	3.82	2019	2022	
pluripotent stem cells	2015	2.72	2019	2020	
age-related macular degeneration (amd)	2017	2.71	2019	2021	
rare	2019	2.57	2019	2021	
visual impairment	2020	2.99	2020	2021	
injection	2020	2.73	2020	2023	
retinal diseases	2019	3.89	2021	2022	
drug-delivery	2021	3.08	2021	2022	
neovascularization	2017	2.99	2021	2023	
geographic atrophy secondary	2021	2.64	2021	2022	
complement system	2022	3.15	2022	2025	
progression	2019	2.71	2022	2025	
stargardt disease	2022	2.69	2022	2023	
vision	2022	2.52	2022	2025	
anti-vegf therapy	2022	2.52	2022	2025	
intravitreal	2023	3.06	2023	2025	
in vivo	2023	3.06	2023	2025	
eye	2017	2.76	2023	2025	
clinical trials	2020	2.66	2023	2025	
fundus autofluorescence	2023	2.63	2023	2025	

Figure 3: Conducting burst word analysis of the literature in the recent decade by means of CiteSpace.

The Figure 2 indicates that over the past decade, AMD caused by oxidative stress and gene therapy vectors and delivery methods have been the main research directions.The buzzwords that are highly relevant to this review and will continue to be prominent until 2025 include the complement system, anti-VEGF therapy, and intravitreal treatment. This confirms that the current research on AMD treatment

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mainly focuses on two approaches: the simple injection of anti-VEGF drugs and the treatment targeting the complement system.(Figure 3)

In order to solve these problems, the author believes that gene therapy will become a more ideal method. At present, in the relevant research of gene therapy for complement, gene enhancement has started the earliest and carried out the most research. Although no drug has been approved in clinical practice, it has also achieved certain phased results. Ongoing trials include the membrane binding inhibitor RAAV-CD59, which targets the formation of membrane attack complex (MAC). CD59 is a naturally occurring membrane binding inhibitor of MAC formation, and MAC levels are elevated in the choroidal blood vessels and retinal pigment epithelium (RPE) of AMD patients. But there are also many trials that have ended in failure, such as Eculizumab, a complement protein C5b inhibitor, Lampalizumab, a complement factor D inhibitor, and the hCFI product GT005. To fully achieve the optimal inhibitory effect, all three pathways of complement activation should be blocked and all active products in the complement pathway, including C3a, C3b, C5a, C5b production or function should be blocked. Blocking only the replacement complement pathway has limited effect and may result in failure of clinical trials. But at the same time, the complement system is an important defense system of the human body, the core of innate immunity, and an important bridge between innate immunity and adaptive immunity, maintaining a delicate balance between activation and regulation. Therefore, the development of complement drugs is very difficult, and how to find a balance between inhibition and activation is the key.

At the same time, the achievements of gene editing are still mainly in the animal experimental stage, and the number of relevant studies is not large. The selected targeted genes are still mainly VEGF and ARMS2/HTRA1, and the main observational indicators are CNV, ROS, SOD2 and so on. At present, the research mainly focuses on delivery vector, reduction of off-target probability and editing efficiency balance. In 2022, an experiment showed that CRISPR-Cas9TX and CRISPR-Cas9 targeting Vegfa in the treatment of AMD mouse models can effectively inhibit laser-induced neovascularization[21].

The occurrence of this situation has many factors. First, the cause of AMD is complex, and the pathological mechanism has not been fully elucidated. Compared with the genes related to complement regulation, the mechanism related to VEGF gene is more clear, the corresponding therapeutic methods are more mature, and the research vein is clearer. Therefore, the target of gene therapy and gene editing in the treatment of AMD is selected. It is normal to preferentially select VEGF; Second, complement drug development is difficult. On the other hand, relevant research is limited by some current technical difficulties of gene editing technology, such as safety (such as the possibility of chromosome translocation, carrier integration or a large number of deletions), and effectiveness. At present, improving the accuracy, efficiency and specificity of gene editing is still one of the focuses of current research. Antibody drugs targeting complement related targets have been put into clinical use. Gene enhancement and other gene therapy methods have opened up a new world for the treatment of AMD. It is believed that in the future, with the development and improvement of technology, more in-depth pathological mechanism research and precise gene editing technology will provide new hope for the research in the field of AMD.

4. Gene editing

Gene editing refers to the specific alteration of gene sequences using enzymes (especially nucleases) to cut DNA strands, remove existing DNA, or insert new DNA segments. It can also involve replacing parts of existing DNA with desired DNA sequences. Homologous recombination technology is the earliest gene editing technique, based on the principle of introducing an exogenous target gene into recipient cells. Through homologous sequence exchange, the exogenous DNA fragment replaces the gene at its original locus. However, this method has a low recombination rate in higher eukaryotes, making it less suitable for large-scale applications. In contrast, gene editing technologies based on DNA nucleases have developed rapidly, addressing this issue. From the first-generation DNA nuclease editing system ZFN, the second generation TALEN, to the third generation CRISPR/Cas9 system, gene editing efficiency has continuously improved while costs have gradually decreased.

4.1. Zinc Finger Nuclease and Transcription Activator-Like Effector Nuclease

Zinc Finger Nuclease (ZFN) technology consists of a DNA-binding zinc finger protein region (responsible for recognizing DNA loci) and the nuclease active region of the restriction endonuclease FokI. Transcription Activator-Like Effector Nuclease (TALEN) technology combines transcription

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activator-like effector proteins (TALE), which specifically recognize DNA sequences, with the nuclease FokI. The design and preparation of the first two generations of technology are relatively difficult and complex compared to the CRISPR/Cas9 system, and their editing efficiency is lower.[22]

4.2. CRISPR-Cas system

The CRISPR-Cas system, which stands for Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated proteins (Cas), serves as the natural immune system of prokaryotes to defend against external viral attacks. This system consists of CRISPR sequences and the Cas gene family. The CRISPR consists of highly conserved repeat sequences and interspaced sequences, with the repeat sequences typically ranging from 20 to 50 base pairs and containing 5 to 7 base pairs of palindromic sequences. The transcription products form crRNA, which can create a hairpin structure, stabilizing the secondary structure and binding with Cas proteins to exert their function. The interspaced sequences are the exogenous DNA sequences captured by the bacteria. The Cas gene family, which includes highly conserved CRISPR-associated genes (Cas genes), encodes Cas proteins that cut foreign nucleic acid sequences. Based on differences in how Cas proteins cut exogenous nucleic acid sequences, the CRISPR-Cas system is divided into two classes. The first class comprises effector complexes formed by multiple Cas proteins, such as Class I, III, and IV. The second class consists of single Cas proteins, such as Class II Cas9 and Class V Cpf1 proteins.

Scientists have adapted the type II CRISPR-Cas9 system into an efficient genome editing tool. In nature, when certain bacteria or archaea are invaded by viruses, the Cas1 and Cas2 proteins participate in capturing a small segment of the viral gene, which is stored in the CRISPR sequence of the DNA. The captured viral gene is known as the spacer sequence. When the same virus invades again, the CRISPR locus, induced by the foreign genetic material, transcribes pre-crRNA (the precursor of crRNA). Simultaneously, the CRISPR-Cas locus upstream expresses tracrRNA (trans-activating crRNA). Pre-crRNA forms a double-stranded RNA through base pairing with tracrRNA, which assembles into a complex with the Cas9 protein. This complex is then processed with the assistance of RNAse III (RNase III), resulting in a short, mature crRNA that includes a single type of spacer sequence RNA and part of the repeat sequence region. The complex formed by crRNA, Cas9, and tracrRNA scans the exogenous DNA sequence, recognizing the original spacer sequence (PAM) that is complementary to crRNA, thus localizing to that region. Subsequently, the Cas9 protein cleaves the site three nucleotides upstream of the PAM. The HNH domain cuts the DNA strand complementary to crRNA, while the RuvC domain cleaves the other non-complementary DNA strand, ultimately causing double-strand breaks (DSB) that form blunt-end products. Unlike the natural CRISPR-Cas9 system, gene editing technology uses artificially designed sgRNA (guide RNA), which fuses tracrRNA and crRNA into a single-stranded guide RNA to recognize the target genomic sequence. After the DNA double-strand break, the DSB can activate DNA damage repair via non-homologous end joining (NHEJ) or homologous recombination (HR), thus achieving the goal of gene modification. [23]

4.3. Base Editor and Prime Editor

Single nucleotide variants account for approximately two-thirds of human genetic diseases and are the genetic basis for many important shape variations in plants and animals. Therefore, developing a precise technology for single-base replacement is crucial. David Liu's team has developed three editors: Cytosine Base Editor (CBE), Adenine Base Editor (ABE), and Prime Editor. They operate without the need for DSB generation or the involvement of donor DNA. By fusing cytidine or adenosine deaminases with catalytically impaired Cas9 (dead Cas9, dCas9), they can facilitate targeted conversions of C:G to T:A or A:T to G:C base pairs.[24]

The first reported BEs are Cytosine Base Editors (CBEs), which conduct targeted C•G to T•A conversions. These consist of cytidine deaminases fused with catalytically impaired Cas enzymes and uracil glycosylase inhibitors (UGI). In a typical CBE, the catalytically impaired Cas enzyme first binds to a specific genomic locus without producing DSBs. Base pairing between the guide RNA and the target DNA strand exposes a single-stranded DNA bubble, which can undergo deamination by the fused cytidine deaminase domain. Since the fused cytidine deaminase is specific for single-stranded DNA substrates, deamination is limited to a small window within the exposed DNA strand. The deamination of cytidine produces uracil, which is protected from base excision by the fused UGI part, leading to U•G mismatches at the target DNA locus.

Adenine Base Editors (ABEs) execute A•T to G•C conversions through a similar mechanism. Since

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no known natural enzyme catalyzes the deamination of deoxyadenosine, which is necessary for adenine base editing in DNA, all described ABEs to date are derived from laboratory-evolved deoxyadenosine deaminases fused with catalytically impaired Cas enzymes. ABEs are particularly useful base editors because they reverse the most common type of pathogenic point mutations (C•G to T•A), which account for about half of the known pathogenic single nucleotide polymorphisms (SNPs). [25]

Prime editing is a cutting-edge genome editing technology that can precisely and specifically introduce all 12 possible point mutations (i.e., 6 types of possible base pair substitutions), small insertions, and small deletions, while exhibiting good editing efficiency. The prime editor is a fusion protein composed of the Cas9 nuclease domain (inactive HNH nuclease) and an engineered reverse transcriptase domain. Prime editors can install point mutations at sites far from the Cas9 cleavage site (＞30 bp), providing greater targeting flexibility compared to nuclease-mediated HDR using ssDNA donor templates, which typically struggle to effectively introduce edits more than 10 bp away from the cleavage site. In principle, this characteristic also reduces the limitations imposed by PAM availability on prime editing, while the off-target probability is lower than that of other methods.[26]

4.4. The application of gene therapy in other genetic eye diseases

At present, the application of gene therapy in genetic eye diseases is mainly in the three categories of LCA, Stargardt and RP. In 2017, FDA approved the AAV gene therapy developed by Spark, which is to introduce normal RPE65 gene into the affected retinal cells. After early clinical treatment, the patient's vision was greatly improved, but later follow-up found that the patient's vision decreased and there was resistance to the imported gene. Therefore, Simply introduce normal genes into abnormal cells in the short term effective method but can not be completely cured, then the use of gene editing abnormal cells abnormal gene editing into normal is one of the effective ways to treat LCA2 type. In recent years, a study used lentviral vector (LV) to successfully introduce adenine single base editor (ABE) into the LCA mouse model, successfully repair the pathological genes in the mouse model, and at the same time, the mouse vision was restored, and the off-target effect often appeared in CRISPR/Cas9 was not significantly observed[27]. For LCA10, Editas Medicine, a company founded by Zhang Feng's team, has introduced EDIT-101 therapy and has entered clinical treatment, restoring sight in many patients. The treatment uses AAV5 vector to deliver saCas9 and CEP290-specific gRNA to the retina to restore the normal expression of the CEP290 gene. This approach involves directly deleting or inverting the mutated intron region by targeting the upstream and downstream parts of the mutated intron region with two GRnas, respectively, and ultimately restoring vision to the patient's eye[28].

Current treatments for Stargardt include vision cycle modulators such as ALK-001, fenretinide, A1120, as well as gene supplement therapy and RPE cell layer cell transplantation with iPSC[29]. STG-001 has also entered phase II clinical trials. The most common type of disease is Stargardt disease Type 1 (STGD1), an autosomal recessive disorder caused by a mutation in the ABCA4 gene. In 2023, Riedmayr LM, Hinrichsmeyer KS et al injected mRNA transsplice reconstruction (REVeRT) into a mouse model of Stargardt disease in the glass. This method can reconstruct full-length ABCA4, but it has not been applied in clinical experiments yet. An experiment began in 2023 to study Stargardt disease (SD) subjects with NTXMCO-004 (STARLIGHT, NCT05417126). In the study, all six enrolled subjects received McO-010, an ambient light-activated multisignature opsin (MCO) gene loaded in adeno-associated virus serotype 2 (AAV2) vector, via intravitreal injection (IVT). The above data is derived from the Non-interventional Long Term Follow-up Study of Participants Previously Enrolled in the STARLIGHT Study (SUSTAIN) NCT06048185.

For RP, similar to the current treatment of AMD, which mostly focuses on anti-VEGF to delay angiogenesis, the current treatment adopts symptomatic treatment to delay the disease process. Gene therapy is also gradually being applied to retinitis pigmentosa (RP). For example, autosomal recessive types of RP can benefit from gene supplementation therapy. While CRISPR/Cas9 has not yet been formally used in clinical practice, it primarily targets P23H, rd10, Rho-KO, and P347S[30]. The subretinal injection of rAAV in the RST-001 Phase I/II Trial for Advanced Retinitis Pigmentosa (NCT02556736) can show that the development of RST-001 in relation to gene therapy is a novel gene therapy application in optogenetics, and phase I and II clinical trials of this drug are expected to be completed in September 2024. hPDE6A rejuvenates the rod-like phosphodiesterase, reduces the increase of intracellular calcium inflow, and prevents the overactivation of the cell death pathway, from PDE6A Gene Therapy for Retinitis Pigmentosa (Pigment)NCT04611503.

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5. Conclusion

With the rapid development of science and technology, our understanding of AMD is deepening, at the same time, gene editing technology is also ushering in unprecedented opportunities. This paper collects a large number of domestic and foreign research data, hoping to provide readers with a comprehensive and in-depth understanding by summarizing the mechanism of complement inheritance and the related progress of gene editing technology. More importantly, we look forward to the potential role of gene editing technology in the field of AMD, and believe that it will bring new hope for future treatment methods, and help us better cope with this serious threat to human vision health challenges.

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