The hereditary abnormalities and genetic therapy of BCM

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Abstract. Blue cone monochromia is a visual disorder caused by genetic mutation, which is a single gene genetic disease thus very suitable for gene therapy. With this technique, mutations in patients' opsin genes can be repaired, thereby restoring their visual recognition of red and green. Compared with traditional treatment methods, gene therapy has obvious advantages in improving curative effect and is expected to achieve a radical cure of the disease. However, gene therapy still faces some challenges and limitations, such as technical validation, cost and accessibility issues. This review focuses on the progress of gene diagnosis and treatment technology in recent years and its application to blue cone monochromatic diseases. Further research and clinical practice will show more support and validation for the development of this treatment, thus providing patients with better treatment options.

Keywords: BCM; genetic therapy; opsin; OPN1LW; OPN1MW.

1. Introduction

Vision is essential to our daily lives, however, some genetic mutations can lead to loss or defects in visual function. In this regard, it is particularly important to understand the genetic background and therapeutic advances in blue cone monochromacy (BCM), a rare visual disorder. BCM is a hereditary visual condition that impairs a person's ability to see colors. Red and green cones in the retina, which are necessary for color vision, are absent in people with BCM [1]. As a result, they can only perceive shades of blue. The severity of BCM varies from person to person, with some people experiencing more severe color vision impairment than others. Common clinical manifestations of BCM include decreased visual acuity, poor color discrimination, and sensitivity to bright light. In this review, we will first explore the structure and function of opsin, which is an important component of vision formation. Next, we will delve into the genetic background of BCM-related diseases, including the relationship between mutated genes and disease development. Finally, we will discuss the importance of gene therapy in BCM and the latest therapeutic advances. It’s better to understand the nature and genetic mechanisms of BCM and provide guidance for future research and treatment through discussion of these recent development in this disease.

2. The etiology of BCM

Abnormalities in opsins' function can lead to visual defects and disorders, one of which is BCM, a condition associated with opsins. BCM is a hereditary visual defect in which patients can only perceive blue light, while their ability to perceive red and green light is severely impaired or nonexistent. Studies have shown that patients with BCM have genetic mutations or deletions in their opsin genes, resulting in structural and functional abnormalities in the protein part of the opsins. These abnormalities further affect the formation of the opsin complex and the normal progression of visual signaling, leading to the occurrence of BCM.

Abnormal opsin function due to genetic mutations is a disease that causes visual disorders, the most common of which is BCM. BCM is a genetic disorder that affects about 1 in 100,000 to 1 in 200,000 people [1]. The disease usually begins in childhood. The visual impairment of patients with blue-cone monochromatism is mainly manifested by severe deficits in red and green colors. They can only see blue and yellow and cannot distinguish other colors. This is caused by mutations in the opsin structure, specifically those associated with the blue photoreceptor opsin (S-cone opsin)[2]. These

mutations cause blue photoopsin to be unable to properly perceive red and green light and convert it into nerve signals.

Clinically, patients with blue cone monochrome vision may also have other visual abnormalities, such as reduced sensitivity, vision loss, and hypersensitivity to glare. Symptoms and severity can vary among patients, but generally affect their ability to function and communicate visually. Therefore, abnormal opsin function caused by gene mutation is the root cause of blue cone monochrome vision.

3. Composition,Structure and functions of Opsins

Opsin, a protein present in the retinas of the eyes, plays a crucial role in visual signaling. Understanding the structure and function of opsins is significant for comprehending the visual process and its related defects and disorders.

Opsins primarily consist of two components: a protein part and an accessory pigment part. The protein part of opsins is a polypeptide chain consisting of approximately 350 amino acids. It contains several conserved structural domains and sites responsible for binding and facilitating visual signaling in conjunction with the accessory pigment. The accessory pigment of opsins is a special molecule called retinal or visual retinol. Retinal binds to a specific site on the protein part through a covalent bond, forming a complex with opsin. This covalent bond is achieved through a condensation reaction between the hydroxyl group of retinal and the lysine residue of the protein part. The formation of the opsin complex is a crucial step in the visual signaling process.

Opsins are family members of G protein-coupled receptors (GPCRs) with seven transmembrane spans that play a crucial role in the process of vision [3]. These proteins have different molecular weight sizes, the most well-known of which is Rhodopsin, which has a molecular weight of about 40kDa. Opsins are composed of several domains, including the N-terminal exocytoplasmic ring, seven transmembrane regions, and a C-terminal cytoplasmic region. The N-telocytoplasmic ring is involved in the localization and polymerization of opsin, seven transmembrane regions are the key parts of the interaction between opsin and the cell membrane, and the C-telocytoplasmic region is involved in the process of cell signal transduction.

The signaling pathway of opsin is an important in the visual system, which is responsible for converting visual stimuli into neural signals and transmitting them to the brain for image processing and cognition. The signaling pathway of opsin mainly includes visual receptors and opsin activation. After light enters the eye, it receives light stimulation through opsin receptors (rods and cones) in the retina, which will undergo conformational changes and activate opsin molecules. The activated opsin molecules bind to G proteins to form activated complexes, further activating phospholipase C to produce secondary signaling molecules, such as inositol triphosphate IP3 [4-5]. They are capable to activate ion channels in retinal cells, resulting in potential changes and transmitting through a network of neurons in the retina and eventually to the optic nerve fibers. The optic nerve fibers transmit nerve signals to the visual cortex of the brain, triggering the integration and processing of visual information, and ultimately forming the visual image.

The whole process of opsin signaling pathway involves the interaction and signal transmission of multiple proteins, from light stimulation to the transformation of nerve signals, which provides us with the basis of visual perception. There are several genes that code for opsin, the best studied of which is the RHO gene, which encodes the protein of opsin, rhodopsin. In addition, there are other opsins coding genes, such as red-green binding protein coding genes OPN1LW and OPN1MW [4].OPN1LW encodes L-opsin, and OPN1MW encodes M-opsin. The two genes are located in the Xq28 region of the X chromosome, and the two genes are adjacent, tandem and highly similar, about 98% of the DNA sequence is the same [6]. In addition, both OPN1LW and OPN1MW genes contain 6 exons, and the sequence of exons 1, 3 and 6 of OPN1LW and OPN1MW genes is completely consistent through CDS region comparison, with only a few base differences on exons 2, 4 and 5. Because of their highly similar structures and adjacent locations, red/green opsin genes are prone to unequal homologous recombination. And the disease is mainly passed on to future generations

through X-linked inheritance. Under normal circumstances, red and green cones sense red and green light signals through opsin encoded by the OPN1LW and OPN1MW genes to produce corresponding color perception. However, people with blue-cone monochromaticity vision have mutations in these two genes, resulting in an inability to perceive red and green light signals properly. These mutations can occur at different sites in opsin, and mutations at some of these sites are very common.

4. The mechanisms of genetic mutations and disease progression of BCM

BCM is mainly caused by mutations in genes responsible for producing red and green cones. Specifically, blue-cone monochromatism is associated with mutations in the OPN1LW and OPN1MW genes. These mutations can be divided into several categories, such as missense mutations, nonsense mutations, deletion mutations, etc.

4.1. Deletion

There are two main cases about gene deletion [7]. The first is the absence of locus control region (LCR), which leads to the failure of gene expression. Normal L and M opsin genes require LCR to regulate their normal expression. LCR is a specific DNA sequence that is present before and between each gene in the opsin gene array. LCR contains specific promoter and regulatory binding sites, which can interact with transcription factors to regulate the transcription and expression of opsin genes. When a deletion occurs in LCR, LCR loses its normal structure and function, resulting in transcription factors that cannot properly bind LCR. The L and M opsin genes cannot be properly regulated and expressed. In the second case, the entire exon (exon 4) of a single opsin gene (L) is missing, causing the opsin to fail to fold into its normal structure, resulting in the opsin being unable to accept light signals normally.

4.2. Non-homologous recombination

Non-homologous recombination results in the reduction of the number of L and M opsin genes to one [3]. When the L and M opsin genes are reduced to only one, it means that only one type of opsin is working properly, which causes the photoreceptor cells in the retina to be unable to effectively perceive red and green light, which in turn affects the perception of red and green photoreceptors, resulting in the occurrence of blue cone monochrome vision.

4.3. Mutation

The first class is a single red pigment gene mutation (Arg247-to-Ter), which occurs in the receptor active region of opsin [8]. Arg247 is an amino acid residue in the active region of the opsin receptor that is mutated to a stop codon (Ter), leading to early termination of the protein. This mutation affects the function of the red and green receptors (L-opsin), and patients have a reduced ability to distinguish between red and green in vision. They usually can't accurately perceive red and green.

The second type is a single 5' red-3 'green hybrid gene mutation (Pro307-to-Leu), which occurs in the GCPR (G protein-coupled receptor) structural functional domain of opsin [3]. With this point mutation, weak interaction between opsin and G protein results in blockage of the photoconduction signal pathway, thus affecting the normal signal transmission of the visual system.

The third and most common type of point mutation is the substitution of arginine for cysteine, which occurs at position 203 (C203R). When this mutation affects the cysteine in rhodopsin, it interferes with the pigment's proper folding and causes severe early retinitis pigmentosa [8]. It is unknown how the C203R mutation affects cone cells, despite the fact that it has been linked to a loss of function in cone cells with color vision abnormalities. The study's findings demonstrated that the patients' cone densities were much lower than those of the normal and color-deficient controls, and that both patients' cones had damage and a weaker outer nuclear layer.

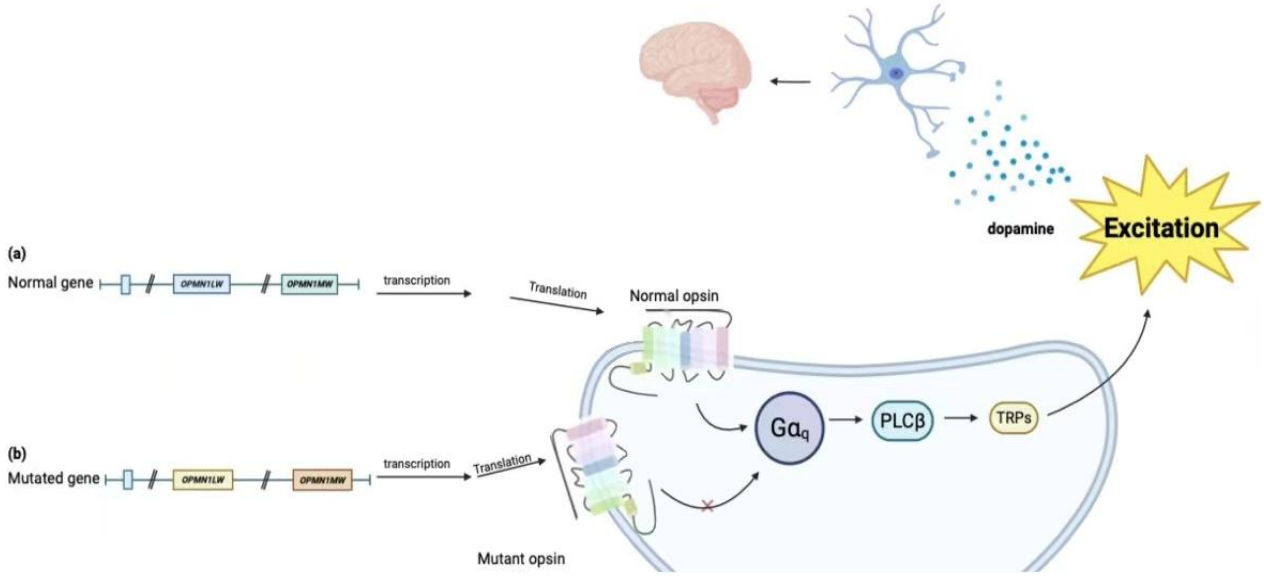


Fig 1. The signal pathway of opsin expression and the signal blockage caused by opsin mutation [9].

Opsin signaling pathway in normal human. Opsin molecules are located on the membrane of retinal cones and undergo conformational changes in response to light. The activated opsin binds to the G protein (opsin transuclein), resulting in the activation of a variety of intracellular signaling molecules. Finally, the excitation signal is transmitted through the neuronal level, from the retina to the cerebral cortex, enabling the perception and processing of vision. (b) Opsin signaling pathway in patients with blue cone monochromatism. In this disease, opsin is mutated, causing its conformational change to be blocked. As a result, light cannot properly activate opsin and bind it to the G protein. This change obstructs the normal process of signal transmission, so the patient cannot perceive the blue light. Compared with the normal people, the opsin signaling pathway of the patients with blue cone monochrome vision is abnormal, which leads to the occurrence of visual impairment.（Some of elements from the image are from https://www.biorender.com/）

5. The progress and challenge of genetic therapeutic in BCM

BCM is a visual disorder characterized by an inability to perceive red and green, and a reliance on blue light for visual perception, mainly caused by mutations in the genes responsible for the red and green cones [7]. Understanding the clinical manifestation, genetic mechanism and genetic pattern of BCM is essential for accurate diagnosis, rational treatment and comprehensive treatment. In recent years, with the rapid development of gene diagnosis and treatment technology, people have a deeper understanding of diseases caused by gene mutations, and some new important discoveries have been made. Among them, gene therapy is considered to be a very potential method for the disease of blue cone monochroma.

Gene therapy is a type of therapy that uses genetic engineering techniques to repair or replace damaged genes. For patients with blue cone monochromatopia, the abnormal opsin function caused by the gene mutation can be repaired by gene therapy. Recent studies have found that by introducing the normal blue photoreceptor gene into patients' retinal cells, they can restore their visual recognition of red and green. According to a new report, there are two possible genetic treatments strategies.

The first approach [3, 10] is gene enhancement, which restores the expression of the mutated gene by delivering a functional copy of a gene via AAV. This can be done by introducing the normal BCM gene into the patient's cells in place of mutated gene expression. It’s common to use an embryonic stem cell line to carry the *OPN1LW* gene and insert a gene trap into intron 2, generating a chimera mice. A promoter-free lacz gene, a terminator symbol, a neomycin resistance gene, an internal ribosome entry site sequence, and a splice receptor sequence make up a gene trap. PCR and

sequencing were used to pinpoint the precise position of the trap. After that, OPN1LW $^{-/-}$ mice were created by crossing the chimeric mice with wild-type mice. Second, the AAV vector is used to create an appropriate AAV vector. It has been demonstrated that this promoter directs transgenic expression to mammalian L/M cone cells. Mouse M-opsin is expressed by an AAV5 vector under the control of the pyramid-specific promoter PR2.1, was then injected under the retina of one eye of *OPN1LW* $^{-/-}$ mice.

The second approach [11] is gene-editing technology, which is designed to directly edit and correct mutations. For example, single-nucleotide gene editing is used to correct mutation sites in *OPN1LW* and *OPN1MW* genes. Homologous recombination mediated gene editing using CRISPR/Cas9 and TALEN techniques. Single guide RNA specially designed for the mutation sites in *OPN1LW* and *OPN1MW* genes was designed to guide the Cas9 protein to precisely cut the target gene. Cas9 protein cleavage produces a double-strand break (DSB), which induces the cell to undergo the DNA repair process. This can lead to two types of repair processes, one non-homologous end joining and the other homologous recombination repair. In order to improve the correctness of repaired genes, it is necessary to add inhibitors of NHEJ and activators of HDR, and use the self-repair mechanism in vivo to synthesize correct *OPN1LW* and *OPN1MW* genes using imported single-stranded oligodeoxynucleotides as templates.

BCM gene therapy research has successfully tested gene augmentation techniques using mice models that mimic big deletion mutations. Compared to traditional treatment methods, such as visual AIDS or color-aided adjustments, gene therapy has brought significant improvements in patients with blue-cone monochrome vision. While traditional treatments usually only relieve the symptoms, gene therapy, which targets the cause directly, promises to cure the disease completely. At present, the genetic diagnosis and treatment of blue cone monochrome vision is still in the experimental stage, so we need more time and effort to ensure its safety and effectiveness. Nevertheless, we are optimistic about the potential of future genetic therapies for the treatment of blue cone monochrome vision.

Genetic diagnostics have great potential to provide new treatment options for inherited diseases that cannot be addressed by conventional treatments. For diseases such as blue cone monochroma, the traditional treatment is mainly through optical assistance or behavioral intervention to improve the patient's visual function. However, these methods can only provide temporary help and do not really repair damaged genes.

The potential advantage of genetic therapy is that it can directly repair or replace damaged genes in patients, thereby restoring visual function. With technologies such as CRISPR-Cas9, researchers can precisely edit gene sequences in cells to fix mutations that cause blue-cone monochrome vision.

However, genetic therapies still face many challenges. First of all, gene editing technology is still in the early stages of development and still needs to be further improved and optimized. Especially in applications targeting human diseases, we need to ensure the accuracy and safety of the editing process to avoid potential unintended results.

Secondly, the evaluation and monitoring of the efficacy of genetic diagnosis and treatment is also an important issue. Because blue-cone monochromia is a visual disorder, we need to develop appropriate methods to assess and track visual recovery in patients. This requires close collaboration with clinical experts and researchers to ensure the success of the treatment program.

Finally, the cost and accessibility of genetic therapies also need to be considered. Although the cost of gene editing has declined as the technology has improved, further efforts are needed to make it feasible and affordable for a wider population.

6. Summary

Gene therapy holds promise for patients with blue-cone monochromaticity vision, but there are still some challenges and limitations. At present, the technology is still in the research stage, and the efficacy and safety of the treatment need to be further verified. In addition, the cost and accessibility of gene therapy remains an issue. However, with the continuous progress of gene diagnosis and

treatment technology and the reduction of cost, gene therapy is expected to become an effective treatment for diseases caused by gene mutations such as blue cone monochromism.

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