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Acta Pharmacologica Sinica

REVIEW ARTICLE

Targeting the photoreceptor cilium for the treatment of retinal diseases

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Photoreceptors, as polarised sensory neurons, are essential for light sensation and phototransduction, which are highly dependent on the photoreceptor cilium. Structural defects and/or dysfunction of the photoreceptor cilium caused by mutations in photoreceptor-specific genes or common ciliary genes can lead to retinal diseases, including syndromic and nonsyndromic diseases. In this review, we describe the structure and function of the photoreceptor cilium. We also discuss recent findings that underscore the dysregulation of the photoreceptor cilium in various retinal diseases and the therapeutic potential of targeting ciliary genes in these diseases.

Keywords: retina; photoreceptor cilium; retinal disease; ciliopathy; gene therapy; genome editing technology; stem cell-based therapy; HDAC6 inhibitor

Acta Pharmacologica Sinica (2020) 41:1410–1415; https://doi.org/10.1038/s41401-020-0486-3

INTRODUCTION

Vertebrate retinal photoreceptors are the most abundant sensory neurons of the retina and are arranged in parallel to convert light stimuli into neurological responses, a biological process called phototransduction. Phototransduction takes place in the outer segment, a highly specialised compartment of the photoreceptor [1]. The structure and protein composition of the outer segmentation differ between rods and cones, which are the two types of photoreceptors in the retina, in a way that corresponds to their functional adaption. Rods are responsible for high-sensitive photon capture in dim light conditions, whereas cones are responsible for high-resolution colour vision that operates in bright light, and each photoreceptor relies on different opsins [2]. Studies over the past few decades on the structure, function, and molecular components of the photoreceptor have highlighted the importance of its ciliary features [3].

The photoreceptor cilium is supported by a microtubule-based axoneme backbone that extends from the basal body of the inner segment [4, 5] (Fig. 1a). Defects in the structure and/or function of the photoreceptor cilium caused by mutations in ciliary genes lead to a broad range of retinal disorders characterised by syndromic and nonsyndromic diseases [6]. A growing list of ciliary genes associated with these diseases has been identified, indicating the potential value of targeting ciliary genes for the treatment of retinal disorders. In this review, we outline the structure and composition of the photoreceptor cilium and the different forms of retinal diseases. We discuss the potential therapeutic value of targeting the photoreceptor cilium in retinal diseases.

STRUCTURE AND FUNCTION OF THE PHOTORECEPTOR CILIUM

Photoreceptors are polarised neurons with various specialised subcellular compartments and therefore require unique protein expression and trafficking systems. Each rod or cone photoreceptor contains three parts: an outer segment where the processes underlying vision are initiated, an inner segment that houses the biosynthetic machinery required for protein synthesis, and a synaptic terminal for signal transmission. The outer segment and inner segment are connected by a narrow, so-called connecting cilium, which is analogous to the transition zone of the common primary cilium [1, 7]. The major distinction between the photoreceptor cilium and the common primary cilium is that the photoreceptor cilium contains a very large outer segment packed with membranous discs and an extended transition zone to support the outer segment and functions as a light sensor (Fig. 1a).

The outer segment is characterised by hundreds of light-sensitive membranous discs stacked in a precise pattern. These membrane discs are renewed by the opposing processes of disc morphogenesis and shedding, which similar to the dynamic assembly of the primary cilium [3]. Phototransduction-associated proteins, such as opsins, that localise in the membranous discs are responsible for initiating the chain of molecular and cellular events that underline normal vision [8]. Outer segment components traffic through the transition zone/connecting cilium region either by intraflagellar transport (IFT)/motor-mediated transport along the axoneme or by diffusion through the plasma membrane [3, 9–11]. The transition zone architecture is characterised by Y-links connecting the microtubule doublets to the ciliary membrane, forming a gate and diffusion barrier to regulate protein entry into and protein exit from the outer segment [3] (Fig. 1b).

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Received: 23 April 2020 Accepted: 28 June 2020
Published online: 4 August 2020

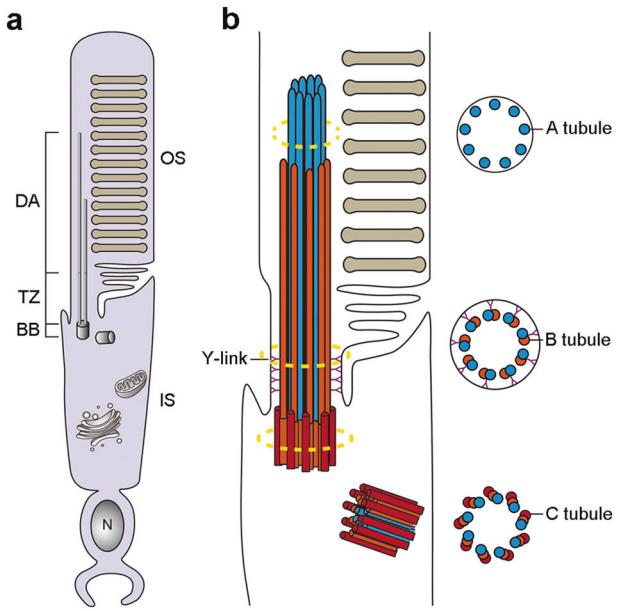

Fig. 1 Structure of the photoreceptor. a Schematic illustration of rod photoreceptors depicting the outer segment (OS), where phototransduction occurs, the inner segment (IS), comprising the Golgi apparatus and mitochondria, the nuclear region and the synaptic terminal. BB basal body, TZ transition zone, DA distal axoneme. **b** Magnification of the photoreceptor cilium and cross-sectional view of the ciliary axoneme.

Ultrastructural cross-sectional analysis of the photoreceptor cilium shows a nine-triplet microtubule arrangement of A, B, and C tubules in the basal body (Fig. 1b). The C tubule is present only in the basal body, while the A and B tubules extend into the transition zone and the proximal axoneme. However, at the distal axoneme, the double microtubules are reduced to singlets that contain only the A tubule [3, 4] (Fig. 1b). Elongation of the axoneme is modulated by anterograde IFT of tubulin subunits by kinesin-2 [12]. Gene-knockout studies in mice have identified an increasing number of proteins responsible for A and B tubule elongation, such as kinesin family member 3 A [13], nephronophthisis 5 (NPHP5) [14], and ADP ribosylation factor-like GTPase 13B (ARL13B) [15].

MUTATION OF CILIARY GENES IN RETINAL DISEASES

Ciliary dysfunction has been implicated in a variety of diseases, which are collectively termed ciliopathies. Due to the ubiquitous nature of cilia, these ciliopathies are syndromic diseases that affect one or more organs, including the retina, central nervous system, cardiovascular system, skeletal system, olfactory epithelium, liver, kidney, gonads, and adipose tissue [16]. Examples of these include Bardet–Biedl syndrome (BBS), Senior-Løken syndrome (SLS), Joubert syndrome (JS), Alström syndrome, Meckel syndrome (MKS), the short rib polydactyly (SRP) group of syndromes, and Usher syndrome (Fig. 2). However, disruptions in ciliary genes have also been identified in a group of nonsyndromic retinal diseases [17]. The most well-known nonsyndromic retinal diseases include retinitis pigmentosa (RP), cone dystrophy (CD), cone-rod dystrophy (CRD), and Leber congenital amaurosis (LCA) (Fig. 2).

Syndromic retinal diseases

Ciliopathies are often multisystemic disorders, in which retinal disease is one symptom. Such retinal disorders are classified as syndromic retinal diseases. JS is a group of genetically heterogeneous disorders characterised by hypotonia, abnormal breathing, developmental delay, ataxia, and retinal dystrophy [18]. JS patients may also present with polydactyly, renal or hepatic defects, an orofacial dysmorphism, with a prevalence rate of 1/80,000–1/100,000 live births [19]. Ocular abnormalities are common in JS patients (~80%), and mutations in a number of genes have been identified (Table 1). Most of these genes encode proteins localised in the basal body, transition zone, or axoneme of the photoreceptor cilium. For example, Abelson helper integration site 1 (AHI1) localises in the basal body and maintains appropriate vesicular trafficking to cilia, which is necessary for the normal function of the outer segment (Fig. 2). Deletion of AHI1 in mice leads to outer segment dysfunction and retinal degeneration [20]. Another ciliary gene, *TMEM237*, encodes a protein localised to the transition zone and is also mutated in individuals with JS [21].

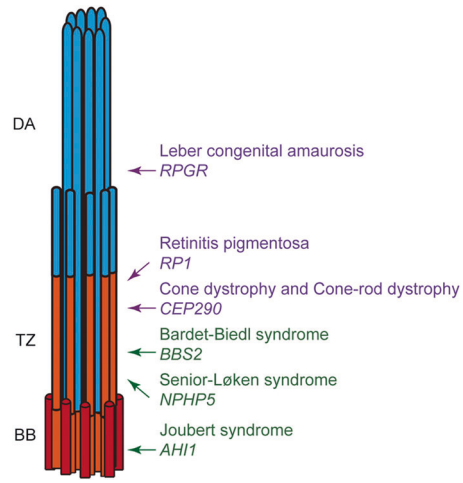

Fig. 2 Mutation of ciliary genes in syndromic (green) and nonsyndromic (purple) retinal diseases. Arrows indicate the localisation of proteins encoded by the ciliary genes. BB basal body, TZ transition zone, DA distal axoneme.

BBS is an autosomal recessive disorder characterised by retinitis pigmentosa, obesity, polydactyly, and hypogonadism, with a prevalence of ~1:100,000 in North America and Europe [22]. Studies indicate that 93% of patients are diagnosed with retinal disorders. To date, a growing list of genes has been identified to cause BBS (Table 1), and many of these genes encode proteins of the BBSome complex, which is a critical adaptor for IFT trafficking (Fig. 2) [9]. In the majority of organisms with BBS mutations, rhodopsin mislocalises to the inner segment and outer nuclear layer, thus reducing its accumulation at an appropriate level in the outer segment. For example, in *BBS2*-knockout mice, rhodopsin mislocalises to the outer nuclear layer and has disorganised membrane stacks in the outer segment, leading to photoreceptor apoptosis and retinal degeneration [23].

SLS is another autosomal recessive ciliopathy characterised by Leber congenital amaurosis in combination with nephronophthisis (NPHP) with a prevalence of 1:1,000,000. NPHP is a cystic kidney disease leading to renal failure. Approximately 10% of NPHP patients also have retinal disorders [24]. To date, several genes have been identified in SLS patients (Table 1), and most of these genes encode proteins that localise in the transition zone of the photoreceptor cilium. For example, the protein encoded by *NPHP5*, a classic SLS-associated gene, localises in the transition zone (Fig. 2) [25]. Deletion of NPHP5 in zebrafish induces the mislocalisation of opsins to the inner segment, suggesting the crucial role of NPHP5 in the transport of proteins to the outer segment [26]. Recently, a well-known ciliary gene, *IFT81*, was also found to be mutated in patients with NPHP-related ciliopathies [27].

Table 1. Representative genes related to retinal diseases.

Syndromic retinal diseases	
Bardet–Biedl syndrome	*BBS1, BBS2, ARL6, TTC8, TRIM32, MKS1, CEP290, WDPCP, IFT27, IFT172, C8orf37*
Joubert syndrome	*INPP5E, AHI1, CEP290, TMEM67, RPGRIP1L, ARL13B, OFD1, TCTN1, TMEM237, TCTN2, MKS1*
Senior-Løken syndrome	*NPHP1, NPHP3, NPHP4, NPHP5/IQCB1, CEP290, SDCCAG8, WDR19, TRAF3IP1*
Nonsyndromic retinal diseases	
Retinitis pigmentosa	*RP1, RP2, RPGR, RP1L1, KIAA1549, IFT140, IFT172, TTC8, REEP6, C2orf71, TOPORS*
Leber congenital amaurosis	*RPGRIP1, CEP164, CEP290, SPATA7, LCA5, IQCB1*
Cone dystrophy and cone-rod dystrophy	*RPGR, RPGRIP, RAB28, C21orf2, C8orf37, CEP78, TTLL5*

These genes are only a small part of genes that have been found to be mutated in patients with different retinal diseases, mainly ciliary genes identified in recent years. For an updated list of genes, please refer to the RetNet website (https://sph.uth.edu/retnet/home.htm).

Nonsyndromic retinal diseases

Mutations in genes encoding ciliary proteins have been found to be causative in nonsyndromic retinal diseases. This class of retinal diseases can be categorised depending on the type of photoreceptors that is principally affected. RP is the most common inherited form of progressive retinal degeneration, which is characterised initially by rod photoreceptor loss, with cone photoreceptors being affected in the advanced stages. It affects up to ~1 in 4,000 individuals worldwide [28]. The symptoms of RP patients typically include blindness, the development of tunnel vision, and a slow, progressive decrease in central vision. X-linked, autosomal recessive, and autosomal dominant are the most common inheritance modes of RP that have been identified [28]. To date, a growing number of associated genes have been identified in RP patients, and many of these genes are ciliary genes (Table 1). For example, *RP1*, a well-known ciliary gene identified in autosomal dominant RP cases, encodes a protein that localises in the outer segment axoneme and is responsible for the correct stacking of outer segment discs (Fig. 2) [29]. Other ciliary genes, such as TOP1 binding arginine/serine rich protein (*TOPORS*, with mutations occurring in autosomal dominant RP), RP1-like 1 (*RP1L1*), *KIAA1549, IFT140* and *IFT172* (with mutations occurring in autosomal recessive RP), and *RP2* and RPGTPase regulator (*RPGR*) (with mutations in X-linked RP), have also been identified [28, 30–33]. Recently, other genes affecting photoreceptor OS development in RP patients have also been identified, such as tetratricopeptide repeat domain 8 (*TTC8*), receptor accessory protein 6 (*REEP6*), and chromosome 2 open reading frame 71 (*C2orf71*) [34–36].

LCA comprises a group of early-onset forms of childhood inherited retinal degeneration with progressive loss of rod and cone photoreceptors and is characterised by vision loss, nystagmus, and severe retinal dysfunction [37]. LCA is rare, with a population frequency of ~1 in 50,000; however, ~20% of children it affects show vision loss [38]. Mutations in ciliary genes affect at least 1/3 of the molecular pathway known to be related to LCA. Among the mutations, centrosomal protein 290 (*CEP290*) and lebercilin (*LCA5*) mutations are the most common causes. *CEP290*, a well-known gene associated with ciliopathy, encodes a large protein localised in the transition zone that is required for ciliary trafficking (Fig. 2) [39]. *LCA5* encodes a protein that also localises in the transition zone of the photoreceptor cilium and interacts with IFT molecules, and it is crucial for IFT trafficking [40]. Other ciliary genes, such as RPGR-interacting protein 1 (*RPGRIP1*), *CEP164*, spermatogenesis-associated 7 (*SPATA7*), and IQ motif-containing B1 (*IQCB1*), have also been identified in LCA patients [6].

CRD comprises a clinically and genetically heterogeneous group of inherited retinal diseases causing early impairment of vision that are characterised primarily by cone photoreceptor degeneration and subsequently by rod photoreceptor loss. The estimated prevalence is 1 in 30,000–40,000 individuals, which makes CRD less common than RP and LCA [41]. This nonsyndromic retinal disease typically presents with the initial loss of colour vision, night blindness, and loss of peripheral visual fields. Depending on the Mendelian inheritance pattern, three types of disease are seen: autosomal recessive (the most common, 60%–70%), autosomal dominant (20%–30%), and X-linked recessive (~5%) [42]. Thus far, a growing list of gene mutations has been identified that give rise to this group of retinal disorders. The proteins encoded by these genes exert different functions in the photoreceptor, especially in the photoreceptor cilium, including phototransduction, outer segment development, and IFT trafficking. For example, *RPGR* encodes a protein that localises in the transition zone of the photoreceptor cilium (Fig. 2) and has been associated with X-linked recessive CD and CRD cases [43]. Other ciliary genes, such as *C21orf2, C8orf37*, tubulin tyrosine ligase like 5 (*TTLL5*), *CEP78*, Ras-associated protein 28 (*RAB28*), and *RPGRIP1*, all encode proteins that localise in the basal body or transition zone of the photoreceptor cilium and have also been identified in patients with CD and CRD [44–46].

TARGETING CILIARY GENES IN RETINAL DISEASES

To date, enormous efforts have been made to develop various therapeutic strategies to protect against or treat retinal diseases. Since mutations in ciliary genes that induce abnormal protein expression are the causative factors for these diseases, restoring normal gene expression using various methods, such as gene therapy, would be a potential effective therapeutic strategy for retinal disorders. Gene therapy, such as gene delivery into cells using lenti- or adeno-associated viruses (AAVs), is currently considered a strategy for the treatment of retinal diseases. To date, gene delivery using AAVs represents the most promising approach to prevent photoreceptor cell degeneration in retinal diseases [47]. The first clinical trial using AAVs was initiated in patients with LCA caused by retinal pigment epithelium 65 (*RPE65*) mutations, and the method used in this study has been approved by the Food and Drug Administration and represents one of the very first successes of gene therapy [48, 49]. Clinical studies have demonstrated that AAVs are safe and effective in clinical therapy, although there have been concerns regarding the immune response to AAVs [50, 51]. There is also a drawback associated with AAV-mediated gene delivery. The AAV virus genome has a packaging limit of 4.7 kb and is thus not suitable for the delivery of large genes, such as *CEP290* [52]. Although alternative AAV

strategies, such as the use of dual or triple AAV vectors, have been used, their efficacy remains to be determined [53, 54].

Genome editing technology based on clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein (Cas), together with an RNA that guides the Cas protein to a predetermined region of the genome, represents an attractive strategy to treat many pathologies, including retinal diseases [55]. Indeed, the CRISPR/Cas system can permanently and precisely replace or remove genetic mutations that cause retinal disease and has been confirmed to repair disease-causing mutations in vivo in RP and LCA patients [56, 57]. For example, in the "rodless" *rd1* mouse model, a well-known preclinical model of RP, CRISPR/Cas-mediated repair of the nonsense point mutation Y347X in the phosphodiesterase 6b (*Pde6b*) gene significantly restored the retinal structure and neurophysiology [56]. However, the use of this technology is confined because of its off-target effects [58]. Combining this technology with the AAV delivery system could reduce the off-target effects. For example, AAV-delivered CRISPR/Cas9 has been used to specifically edit the guanylate cyclase 2D (*GUCY2D*) gene, the mutation of which is the leading cause of CRD and LCA, and a significant effect has been observed in mice and macaques in treating inherited retinal diseases [59]. Therefore, the combination of CRISPR/Cas9 technology with AAV delivery systems represents a novel effective strategy for the treatment of retinal diseases, but the clinical efficiency needs to be determined.

Given the crucial role of the photoreceptor cilium and the large number of ciliary gene mutations that have been identified in various retinal disorders, targeting the photoreceptor cilium and ciliary genes may be a potential approach for the treatment of or protection against retinal diseases.

CONCLUDING REMARKS

In recent years, we have observed tremendous advances in the elucidation of photoreceptor cilium composition and function and the identification of key ciliary modulators. However, the answers to several questions regarding ciliary function in retinal diseases remain elusive. For example, studies have identified several ciliary genes and observed consequent ciliary defects in various retinal diseases. It will be of particular interest to investigate the direct action of the photoreceptor cilium in retinal diseases. It also remains unclear whether cilia in other ocular tissues or cells besides the retina, such as the cornea and iris, and retinal pigment epithelium cells also play a significant role in these retinal diseases. Photoreceptor cilia and the identified gene mutations associated with retinal disorders represent an attractive target for the treatment of retinal diseases; gene-based therapy for LCA caused by *RPE65* mutations has been approved by the Food and Drug Administration [49]. However, it remains to be examined whether similar success can be obtained by targeting other ciliary genes, especially the large gene encoding CEP290. Another promising approach is the use of nanoparticles with a transgene-carrying capacity of up to 20 kb [60]. Unfortunately, no clinical trials have yet been initiated. In addition, the genetic heterogeneity of retinal diseases, combined with the high costs associated with virus generation, must be considered. Thus, further efforts are needed to develop new gene-based strategies for treating retinal diseases.

Recently, stem cell-based therapy for the treatment of retinal disorders has been used to replace damaged or dead photoreceptors with healthy cells. iPSCs are the focus of this approach, since the risk of immune rejection is low because cells are obtained directly from the patients themselves [61]. However, the mutations present in patients are also retained in the iPSCs derived from the patients, which reduces the functionality of transplanted cells. The CRISPR/Cas9 or TALEN techniques can be exploited to correct the mutant genes in patients to allow the use of gene-corrected, genetically matched donor cells for transplantation [62]. This gene editing approach has also been used to repair disease-causing mutations in vivo in the eye [56]. However, for cases of diverse ciliopathies caused by single nucleotide mutations, the base editing efficiency of the CRISPR system is far below that required for therapeutic needs [63].

Corresponding with the growing list of ciliary genes identified in retinal diseases, compounds that target these genes show a significantly protective or therapeutic role in retinal diseases. For example, small molecular compounds that target histone deacetylase 6 (HDAC6), a key regulator driving cilium disassembly, present an attractive target for the prevention and treatment of ciliopathies and retinal disorders [64–66]. HDAC6 is localised at the basal body of the photoreceptor cilium and accumulates in the transition zone in the photoreceptors of oxygen-induced retinopathy mice in a mouse model of retinopathy of prematurity, which causes disassembly of photoreceptor cilia and outer segment dysfunction, ultimately leading to retinal disorders. Moreover, depletion of HDAC6 or inhibition of HDAC6 with small-molecule compounds can significantly protect mice from oxygen-induced pathological changes in photoreceptors, indicating the potential value of HDAC6-targeted agents for the prevention of retinopathy of prematurity. In addition, HDAC6 inhibitors are not toxic to normal tissues, and pan-HDAC inhibitors, such as vorinostat, romidepsin, and panobinostat, have already been approved by the Food and Drug Administration for the treatment of cancer [67, 68]. HDAC6-deficient mice also appear to be superficially normal under standard laboratory conditions [69]. These outcomes suggest that the inhibition of HDAC6 is unlikely to have major adverse effects, although further efforts are needed to design novel and effective HDAC6 inhibitors with high selectivity for treating retinal diseases.

ACKNOWLEDGEMENTS

This work was supported by a grant from the National Natural Science Foundation of China (31701169).

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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