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REVIEW ARTICLE

Clinical manifestation and current therapeutics in X-juvenile retinoschisis

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Abstract: X-linked juvenile retinoschisis (XLRS) is one of the common early-onset hereditary retinal degenerative diseases in men. The common symptoms of XLRS range from mild to severe central vision loss and radial stripes created by the fovea, the division of the inner layer of the retina in the peripheral retina and the significant decrease in b-wave amplitude (ERG). Retinoschisin, the 224-amino-acid protein product of the retinoschisis 1 (RS1) gene, contains a discoid domain as the primary structural unit, an N-terminal cleavable signal sequence, and an oligomerization-area component. Retinoschisin is a homo-octamer complex with disulfide links that are released by retinal cells. It helps preserve the retina's integrity by binding to the surface of photoreceptors and bipolar cells. As a recessive genetic disease, XLRS was usually treated by prescribing low vision aids in most clinical cases. A gene replacement therapy based on adeno-associated virus vectors was initiated and showed a breakthrough in treating XLRS in 2014. Understanding the revolution of gene therapy for treating XLRS may accelerate its development and make this gene therapy the template for developing therapeutics against other inherited retinal diseases.

Keywords: Adeno-associated virus vectors; Hereditary retinal degenerative diseases; X-linked juvenile retinoschisis

1. INTRODUCTION

The eye consists of three layers, sclera, choroid, and retina, each of which lies flat against each other to form the eyeball. Retina forms the innermost layer which lines the back of the eye and consists of two layers: the neurosensory retina and the retinal pigment epithelium.[1] The neurosensory retina is constitutively made up of three groups of neurons: photoreceptors, bipolar cells, and ganglion cells. Photoreceptors contain photopigments which are involved in phototransduction.[1] Bipolar cells are second-order neurons in the visual pathway which transmits signals from photoreceptors to ganglion cells. In order for light to be detected, photons must first travel across the ganglion cells and through the entire retinal depth to reach photon-absorbing photoreceptors located in the distal part of retina.[2] Hence, well-maintained structural integrity and cellular organization of the retina play a pivotal role in giving photons a smooth journey through the eye to be processed into quality images. Globally, hereditary retinal degeneration which encumbers the processing and signaling throughout the eyes represents the most prevalent culprit for eyesight deterioration among childhood and young population.[3]

2. CLINICAL PRESENTATION OF X-JUVENILE RETINOSCHISIS

X-linked juvenile retinoschisis (XLRS) is an early-onset inherited macular degeneration and is estimated to affect 1/5000 to 1/25 000 males worldwide.[4] XLRS is present at birth but often diagnosed later in life because infant children's routine life is normally not disturbed by moderate visual loss.[5] The first diagnosis is usually made at school age when visual impairment interferes with patients' daily activities, especially in reading. Female carriers of the gene of retinoschisis 1 (RS1) mutation typically remain asymptomatic, although X-chromosome inactivation can lead to the expression of functionally defective RS1 protein and presents pathogenic phenotypes in some rare cases.[6,7] Although clinical phenotypes in patients with XLRS are varying, a hallmark consistently observed in all XLRS patients is "foveal schisis." Foveal schisis is characterized by a spoke-wheel pattern of small cystoid spaces which are densely distributed in the fovea and arranged in a stellate or fine radial striae pattern.[4] The splitting of the retina is primarily restricted to inner retinal layers. Although the severity may greatly vary, the majority of patients experience vision loss which remains stable at 20/100 with little to no progression.[5] Over the course of the disease, ≈5% of all affected males may develop severe complications, such as vitreous hemorrhage or retinal detachment.[8]

3. MOLECULAR GENETICS OF XLRS

XLRS develops as a result of mutations in the RS1 gene located on the short arm of X-chromosome (Xp22.1-p22.3). This gene

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encompasses six exons and five introns, spanning a region of 32.43 kb of DNA. The RS1 mRNA transcript is 12 kb in length and translates into a 224-amino-acid (aa) protein, termed retinoschisin.[5] The retinoschisin monomer structurally consist of a 23-aa N-terminal signal peptide which is cleaved off through the secretory pathway to give rise to mature RS1 monomer protein and a 157-aa highly conserved sequence motif called the discoidin. This domain is found in many transmembrane and extracellular proteins and is important for the adhesive function of RS1. Besides, a unique RS1 domain upstream of discoidin domain and a 5-aa-C-terminal segment are crucial for the formation of retinoschisin oligomers. Eight RS1 monomers are linked together by Cys59-Cys223 intermolecular disulfide bonds, forming its functional conformational RS1 octamer.[5,9] A dimer of back-to-back octamers formed by Cys40-Cys40 was also found to exist lately.[10] Disulfide-linked homo-octamerization is implicated in increasing the binding affinity of retinoschisin for its receptor on cell surfaces.[5] Profiling of RS1 expression in various mammalian species detected prominent retinoschisin expression at the extracellular surfaces of the rod and cone inner segments, most bipolar cells, and the two plexiform layers of the retina. Retinoschisin expression was also observed in pinealocytes of pineal gland which share a common neuroectoderm origin with the retina.[9]

Retinoschisin was shown to interact which phosphatidyl serine (PS) of lipid bilayers in a Ca^{2+}-dependent manner and participate in intercellular interactions and cytoskeleton organization.[11] In addition, other interacting ligands may include Na+/K+ ATPase, the sterile alpha and TIR motif-containing protein, and L-type voltage-gated calcium channel.[9] Although there still lacks a confirmative understanding of the function of retinoschisin, it is generally known as a secreted soluble cell-adhesion protein engaged in maintenance of retinal architecture and photoreceptor-bipolar synapse as a largely disarranged retinal structure was invariably observed in XLRS patients and murine X-linked juvenile retinoschisis (Rs1h) knockout (KO) mice. Additionally, on the basis of cystic cavities filled with fluids typical of XLRS patients, it was postulated that retinoschisin may function as a regulator of fluid homeostasis within photoreceptor and bipolar cell layers.[5]

To date, a broad spectrum of >200 pathogenic mutations have been reported in the RS1 gene.[4] Out of these, mutation in the discoidin domain is predominant although they can be found across the entire gene, irrespective of the exons or intervening regions. It is estimated that about 40% are nonsense mutations or frameshift mutations, rendering synthesized RS1 protein nonfunctional. The fate of RS1 protein can be affected by these mutations in multiple ways, such as misfolding and failure to release protein from the endoplasmic reticulum, defective oligomerization or mislocalization.[5]

4. THE CLINICAL DIAGNOSIS AND CURRENT TREATMENT FOR XLRS

Clinical examination fundamentally constitutes the diagnosis of XLRS while imaging and other diagnostic testing have an important role to play in further evaluating disease severity and progression. Electroretinogram (ERG) of patients with XLRS demonstrates abnormalities, although none of them are of determining and differential diagnostic value for XLRS. Although the amplitude of dark-adapted b-wave is reduced, a-wave remains unaffected.[4] Lamellar schisis which is undetectable by a normal clinical check can be revealed by optical coherence tomography (OCT). This imaging testing also allows observing splitting in layers other than the nerve fiber layer.[8] Other retinal function tests such as color vision, electro-oculography may show abnormal results but their diagnostic value is generally insignificant.[12]

Being aware of a family pedigree, any young male at risk for XLRS needs careful clinical observation throughout his life. More heed should be paid to accurately diagnosing those with XLRS, and low vision aids can be implemented as early as possible to benefit them in the long run. In case of retinal detachment, surgical interventions can be considered but quite formidable and fallible.[5] No treatments for XLRS have been clinically proven so far though some medication-based strategies, for example, carbonic anhydrase inhibitors dorzolamide and acetazolamide, have been considered.[13,14] However, none of these measures tackle the rudimentary disease etiology, which is the deficiency of healthy RS1 protein required for normal retinal structure and synaptic connectivity.

As a recessive genetic disease, XLRS is potentially treated with gene replacement therapy. Optimistic signs from in vivo studies on RS1 KO mouse models intravitreally injected by adeno-associated viral vectors Adeno-Associated Virus Vector Serotype 8 (AAV8) and recombinant AAV2 (rAAV2) carrying functional RS1 facilitated and accelerated gene therapy human clinical trials.[8] The National Eye Institute (NEI) is conducting one of the trials (NCT02317887) using AAV8 vector as carrier and the other (NCT02416622) using rAAV2 was initiated by Applied Genetic Technologies Corp (AGTC). However, according to the announcement by AGTC in December 2018, Phase 1/2 clinical trial of rAAV2-based gene therapy for treatment of XLRS failed to show any clinical benefit after six months and these results precluded them from furthering proceeding with this product.[15] Meanwhile, latest report from NEI declared that AAV8 treatment had positive safety and tolerability although further optimization is required for dosage regimens and immuno-suppressive strategies to control immune reactions elicited by the vectors.[16]

5. GENE THERAPY FOR RETINAL DISEASES

Gene therapy in ophthalmology has been growing at an unprecedented rate and manifests massive application potential.[17] Even with several potential hazards pertaining to insertional mutagenesis and resulting unpredictable consequences and hindrances, the gene therapies-driven approaches for treating the genome-based mutation diseases are still provide supportive benefits from innovative research to move forward into the bedside in the offing.[18,19] Notably, based on its anatomical accessibility and the immunologic privilege as well as confinement status enabled by tight blood-ocular barriers, the retina is particularly deemed as a great fit for gene therapy.[20] Over the years, inherited retinal diseases have emerged as one of leading targets for gene therapy research and a growing number of clinical trials are currently ongoing in this field. AAV-mediated gene supplementation for autosomal recessive mutations in the Retinoid isomerohydrolase (RPE65) gene which is associated with Leber congenital amaurosis type II and retinitis pigmentosa successfully restored visual impairment in canine models.[21] Human clinical trials following these successes continued to reap positive results, leading to the first US regulatory approval of a gene therapy (LUXTURNAR; Spark Therapeutics) in January 2018. Inspirational success story of LUXTURNAR blazed a trail for a multitude of AAV-based gene therapy clinical trials targeting other inherited retinal dystrophies, including X-linked retinoschisis,[15] X-linked retinitis pigmentosa, Leber hereditary optic neuropathy, choroideremia, and achromatopsia.[17] Nevertheless, the inability to completely dictate transgene copy numbers and as a consequence, unpredictable expression levels and duration have been widely postulated as major hurdles of such implementation.[22]

In conclusion, XLRS is a rare congenital malformation of the retina associated with mutations in RS1 gene. Although gene replacement therapies for XLRS are going under clinical trials, sustainable therapeutic benefits remain unobtainable and some uncertainties.[15,22] In addition, in attempts to achieve more persistent therapeutic efficacy,[17] it is a potential switch from current gene augmentation to a precision-based genome editing-approached therapeutics in the coming future.

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