Metabolic rescue of cone photoreceptors in retinitis pigmentosa

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Abstract:

Retinitis pigmentosa (RP) encompasses a group of inherited retinal dystrophies characterized by the primary degeneration of rod and cone photoreceptors. It is a leading cause of visual disability, with an incidence of ~1 in 7000 persons. Although most RP is nonsyndromic, 20%–30% of patients with RP also have an associated nonocular condition. The gene mutations responsible for RP occur overwhelmingly in rod photoreceptors. Visual loss frequently begins with night blindness in adolescence, followed by concentric visual field loss, reflecting the principal dysfunction of rod photoreceptors. Although the visual disability from rod dysfunction is significant, it is the subsequent loss of central vision later in life due to cone degeneration that is catastrophic. Until recently, the reason for cone dysfunction in RP was unknown. However, it is now recognized that cones degenerate, losing outer segment (OS) synthesis and inner segment (IS) disassembly because of glucose starvation following rod demise. Rod OS phagocytosis by the apical microvilli of retinal pigment epithelium is necessary to transport glucose from the choriocapillaris to the subretinal space. Although cones lose OS with the onset of rod degeneration in RP, regardless of the gene mutation in rods, cone nuclei remain viable for years (i.e. enter cone dormancy) so that therapies aimed at reversing glucose starvation can prevent and/or recover cone function and central vision.

Keywords:

Cone dormancy, glucose, metabolism, retina, retinal pigment epithelium, retinitis pigmentosa

Introduction

Retinitis pigmentosa (RP) is a group of inherited retinal diseases (IRDs) with variable clinical presentations from mild nyctalopia to total blindness. The disease is rare with an incidence of <1 in 7000 persons.[1,2] RP is the most frequent cause of IRD in adults, although it can also present in early childhood as Leber's congenital amaurosis. The gene mutation can be inherited as an autosomal dominant, autosomal recessive, or X-linked disease, although about 35% of cases in the United States have no other affected family member (i.e., simplex RP) and is referred to as nonsyndromic RP. Retinal degeneration can also occur as a manifestation in syndromes, such as Usher's syndrome (sensorineural hearing loss, vestibular dysfunction) and Bardet-Biedl syndrome (obesity, mental retardation).

Most genetic mutations in RP are expressed in rod photoreceptors, although gene mutations in the retinal pigment epithelium (RPE) may also occur (e.g., Mer-TK). Enormous functional diversity in mutant rod photoreceptor genes includes visual transduction (e.g., rhodopsin), photoreceptor structure (e.g., RDS peripherin), extracellular matrix, retinal development, protein folding/trafficking, protein transport, retinoid cycle (e.g., RPE65), disc shedding, transcription factors, pre-mRNA splicing, photoreceptor cilia, and cGMP channels. Mutations in more than 80 genes have been identified in nonsyndromic RP (https://sph.uth.edu/retnet/sum-dis.htm#A-genes).

Visual loss in RP usually begins with rod degeneration at the equator and presents

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as decreased night vision (i.e., nyctalopia) with loss of peripheral vision, ultimately resulting in tunnel vision. Patients may lose up to 90% of rod photoreceptors before visual changes are noticed by the patient.[3] With progression into the later stages of the disease, cone photoreceptors become dysfunctional compromising central visual acuity and color vision, resulting in functional blindness.

The rhodopsin (*RHO*) gene was the first gene identified causing RP in 1990.[4] More than 24 different mutations in RHO are associated with autosomal dominant RP (adRP), and the Pro23His (P23H) mutation in RHO is responsible for ~10% of all adRP in North America; only a few RHO mutations are associated with autosomal recessive RP.[5] Two classes of adRP-RHO mutations have been recognized clinically.[6] Class A patients lose rod function over the entire retina and have an early onset of night blindness. Since the widespread loss of rod function threatens the survival of central vision, cone preservation is of utmost importance. In contrast, Class B patients have a milder clinical phenotype in which photoreceptor degeneration is heterogeneous and rods have the potential to be rescued so that cone photoreceptors can be preserved.[2]

Although most RP mutations occur in rod-specific photoreceptor genes, leading to progressive rod destruction, loss of peripheral vision, and dark adaptation, it is the subsequent loss of cone function (i.e., cone dormancy and ultimately cone demise) that results in functional blindness. Why should cones become dysfunctional and ultimately degenerate if the genetic mutation is in rods? This conundrum remained unsolved for many years, until recently.

In 2009, Punzo *et al*.[7] noticed that mice treated systemically with insulin had prolonged cone survival, while depletion of endogenous insulin had the opposite effect. They concluded that nonautonomous cone death in RP could, at least in part, be due to the starvation of cones. Since then, the symbiotic relationship between the neural retina and RPE has been explored in more depth.[8] Du *et al*.[9] investigated metabolic uptake and release, as well as metabolic genes, in the human neural retina and RPE. They observed that the neural retina and RPE have distinct but complementary metabolic features. They identified the following key features [Figure 1]:
1. Neural retinal metabolism centers on NADH-related metabolism for ATP production and the synthesis of neurotransmitters such as glutamate and GABA. The neural retina consumes glucose, aspartate, glutamate, citrate, nicotinamide, and other nutrients to sustain its active glycolysis, mitochondrial oxidative metabolism, and neurotransmitter synthesis
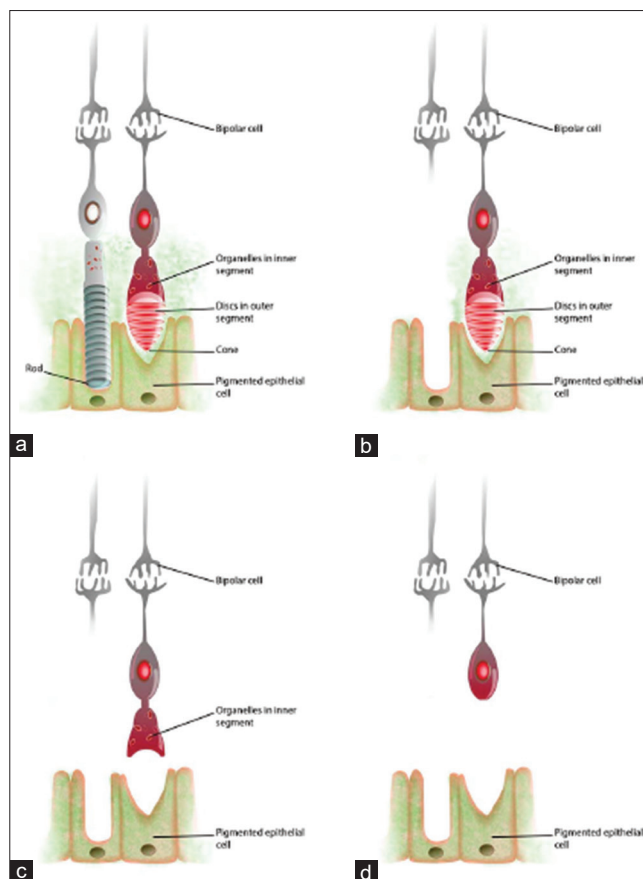

Figure 1: A proposed model of metabolic communications between human retina and retinal pigment epithelium/choroid. This figure was reproduced from Figure 6 in Li B, Zhang T, Liu W, *et al*. Metabolic features of mouse and human retinas: Rods versus cones, macula versus periphery, retina versus retinal pigment epithelium. I Science. 2020;23 (11):101672 and used under the CC BY-NC-ND 4.0 license

2. RPE/choroid metabolism centers on NADPH-related and acetyl-CoA metabolism for energy and biosynthesis. RPE/choroid consumes glucose, proline, nicotinamide, branch-chain amino acids, and other nutrients for pentose phosphate pathway, reductive carboxylation, serine synthesis, one-carbon metabolism, citrate synthesis, ketone body synthesis, and cholesterol synthesis.

Thus, the neural retina and RPE/choroid have tight metabolic communications with complementary profiles in nutrient consumption and energy production.

Since the most common form of adRP in the United States is the P23H mutation in *RHO*, we established a transgenic miniature swine model using the human P23H RHO gene to investigate the mechanism of cone demise. Somatic cell nuclear transfer was used to create six transgenic founders.[10] A single transgene integration site was observed in 5 of the six founders. All founders had abnormal scotopic and photopic full-field electroretinography after 3 months and could be grouped into moderately and severely affected groups.

The offspring of one founder inherited the transgene as an autosomal dominant mutation. mRNA analysis demonstrated that 80% of total RHO was mutant P23H. Thus, this large animal model served as a novel tool for study of the pathogenesis of adRP and is referred to as P23H retinopathy.

Functional studies have detected deficits in retinal signaling in asymptomatic children from families with inherited adRP. Whether retinal abnormalities are present earlier during gestation or shortly after birth in a subset of children with adRP is unknown. Human infants with such aggressive RP might never have had rod vision, but cone vision might be unaffected. Our pig model of P23H retinopathy allowed us to explore this possibility. We tracked changes in prenatal and early postnatal retinal morphology, as well as early postnatal retinal function.[11,12] We observed that prenatal expression of mutant RHO altered the normal morphological and functional development of rod photoreceptors. Despite this significant change, cone photoreceptors were unaffected. Such aggressive forms of RP in preverbal children, as described above for Class A adRP-RHO, would require early intervention to delay or prevent cone degeneration and functional blindness.[2]

Photoreceptors are among the most metabolically active cells and, like other neurons, depend on glucose for energy production and outer segment (OS) synthesis. A previous study in primary culture of cones showed that purified rod-derived cone viability factor (RdCVF) can bind a Glut1 complex on cone inner segments (ISs) and promote glucose uptake.[13] In 2016, we observed that the degeneration and demise of rods in P23H retinopathy resulted in the sequestration of glucose in the RPE, depriving cone photoreceptors of a major metabolite.[14] The resulting glucose starvation impaired the synthesis of cone OSs, and their mitochondrial-rich IS dissociated. Loss of cone OS resulted in both structural and functional (i.e., electrophysiologic) abnormalities in the swine visual streak, which is analogous to the macula in human. We confirmed this conclusion using the subretinal injection of glucose in P23H retinopathy to bypass its retention in the RPE. The resultant regeneration of cone OS and the return of photopic electrophysiologic activity were observed not only in P23H retinopathy but also in other rodent models of RP caused by mutations in other rod photoreceptors or RPE genes.

Cone degeneration with loss of both structural and functional characteristics is referred to as "cone dormancy" [Figure 2].[15] Cone photoreceptor cells survive in this dormant state with little more than a nucleus persisting for years in P23H retinopathy. The reactivation of dormant cones by the subretinal injection of glucose, to bypass its retention in RPE, proved that glucose metabolic

abnormalities were responsible for cone degeneration in RP. Furthermore, we linked retention of glucose in the RPE to diminished mutant rod OS extension, thereby preventing glucose transport to both persistent mutant rods and cones.[14] Once glucose is sequestered in the RPE and cone OS synthesis is lost, RdCVF or genetic enhancement of glucose metabolism in cones through Sirt6[16] is not capable of reinitiating OS synthesis or preventing cone dormancy.

Glucose metabolism in cones is tightly regulated by the thioredoxin-interacting protein gene (TXNIP), through regulation with monitoring of the glucose level within a cell. It functions like a tumor suppressor whose repression in cancer is important in switching glucose metabolism from aerobic to anaerobic (e.g., the Warburg effect).[14] In cones, downregulation of TXNIP, and thus the release of its inhibition of Glut 1, reflects an attempt to increase glucose uptake when available glucose diminishes in RP. Since glucose transport from the RPE to photoreceptors is diminished in RP, attempts at neuroprotection or regulation of metabolic pathways will ultimately fail due to a lack of glucose required for the assembly of cone functional structures. There are two approaches we have shown to address this problem.[14] First, the replacement of glucose in the subretinal space by direct injection. Second, by the subretinal injection of wild-type rod OS that reversed the sequestration of glucose in RPE. Rod OS phagocytosis by apical microvilli on RPE restored the conventional intracellular glucose transport pathway from the basal to apical surface of the RPE cell.

Further investigation of RPE glucose transport from the choriocapillaris to the subretinal space revealed that that contact between externalized phosphatidylserine (PS) on rod OS tips and apical RPE receptors activates Akt, linking phagocytosis of OS tips with glucose transport to photoreceptors for new OS synthesis.[17] As abundant mutant rod OS tips shorten in RP, Akt activation is lost, and the onset of glucose metabolism in RPE with should diminished glucose transport combine to cause photoreceptor starvation, accompanying retinal metabolome changes. Subretinal injection of OS tip mimetics displaying PS restores Akt activation, glucose transport, and cone function in end-stage RP after rods are lost.[17]

A therapeutic option that would provide time for the implementation of definitive reparative intervention in RP, such as gene therapy or retinal transplantation, would forestall mutant rod photoreceptor clearance due to misfolded RHO in P23H retinopathy.[18] If dysfunctional rods could be maintained to serve their secondary function of cone preservation, it would be of significant visual benefit to patients. We found that the induction of a danger-associated molecular pattern (DAMP) "eat me" signal on the surface of mutant rods correlated with
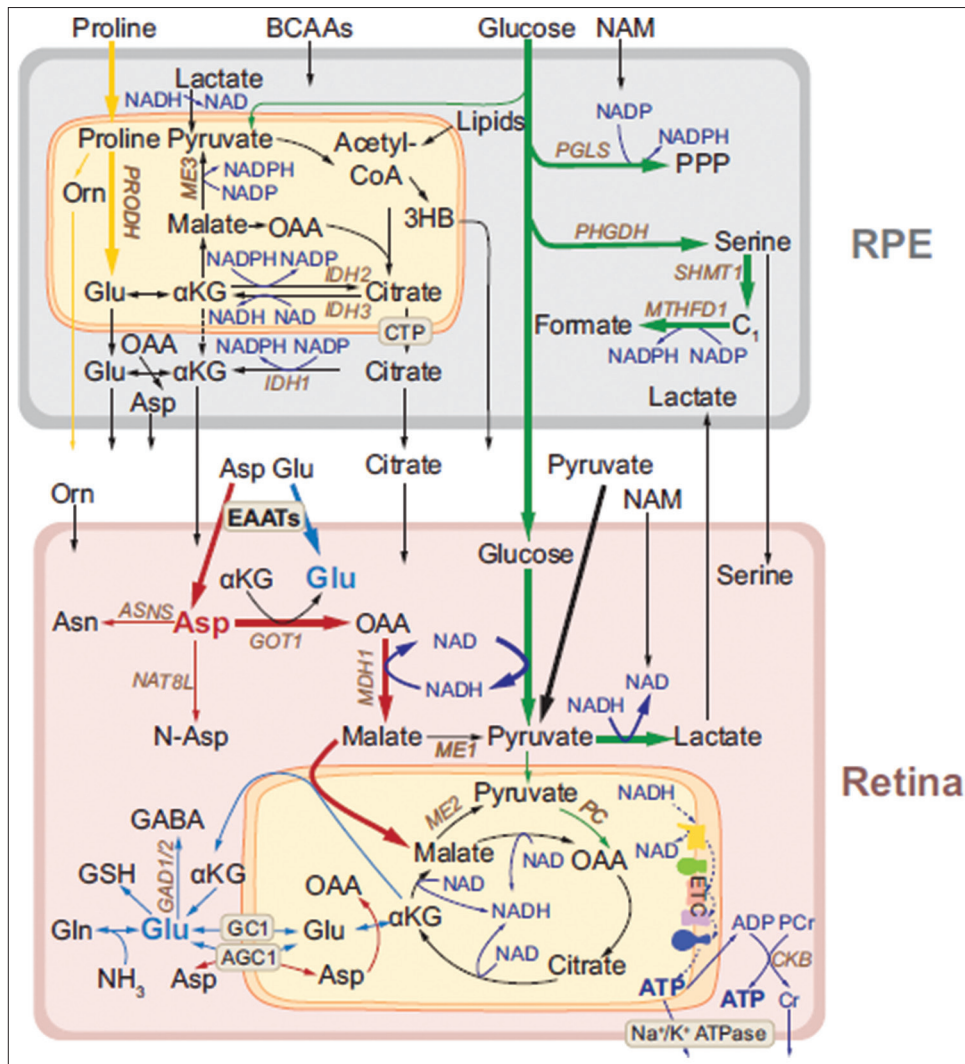
Figure 2: Cone dormancy in retinitis pigmentosa results in viable cone nuclei for many years after outer segment/inner segment degeneration. This figure was reproduced from Figure 6 in Kaplan HJ, Wang W, Dean DC. Restoration of cone photoreceptor function in retinitis pigmentosa. Transl Vis Sci Technol 2017;6 (5):5 and used under the CC BY-NC-ND license

targeting these cells for programmed cell replacement by retinal myeloid cells. Glucocorticoid therapy led to the replacement of this DAMP with a "don't eat me" immune checkpoint on the rod surface and inhibition of programmed cell replacement. Surviving rods continued to promote glucose transport to cones, maintaining their viability.

Conclusion

Glucose starvation of cones occurs in RP because the rod OS is necessary to transport glucose from the choriocapillaris to the subretinal space. Since cones lose OS with the onset of rod degeneration, regardless of the genetic mutation in rods in RP, but cone nuclei remain viable for years after the onset of cone dormancy, therapies aimed at reversing glucose starvation may prevent and/or recover cone function and central vision in RP. Our observations should allow several new

approaches to either maintain or restore central vision in patients with RP until definitive treatment modalities can be implemented.

Conflicts of interest
The authors declare that there are no conflicts of interest in this paper.

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