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Commentary

Retinitis pigmentosa (RP), the most common form of rod-cone dystrophy, is caused by greater than 3100 mutations in more than 71 genes, many of which are preferentially expressed in rod photoreceptors. Cone death generally follows rod loss regardless of the underlying pathogenic mutation. Preventing the secondary loss of cone photoreceptors would preserve central visual acuity and substantially improve patients' quality of life. In this issue of the *JCI*, Wang et al. demonstrate that adeno-associated virus-mediated overexpression of *TGF-β1* promoted cone survival and function in 3 distinct RP models with rod-specific mutations. TGF-β1 induces microglia to metabolically tune from a glycolytic phenotype (M1) to an oxidative phenotype (M2), which associates with neuroprotection and the antiinflammatory ecosystem. Consolidating the results of this study with our current understanding of how TGF-β1 regulates microglia polarization, we highlight cell-specific metabolome reprogramming as a promising non-gene-specific therapeutic avenue for inherited retinal degenerations.

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Precision metabolome reprogramming for imprecision therapeutics in retinitis pigmentosa

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Retinitis pigmentosa (RP), the most common form of rod-cone dystrophy, is caused by greater than 3100 mutations in more than 71 genes, many of which are preferentially expressed in rod photoreceptors. Cone death generally follows rod loss regardless of the underlying pathogenic mutation. Preventing the secondary loss of cone photoreceptors would preserve central visual acuity and substantially improve patients' quality of life. In this issue of the *JCI*, Wang et al. demonstrate that adeno-associated virus-mediated overexpression of *TGF-β1* promoted cone survival and function in 3 distinct RP models with rod-specific mutations. *TGF-β1* induces microglia to metabolically tune from a glycolytic phenotype (M1) to an oxidative phenotype (M2), which associates with neuroprotection and the antiinflammatory ecosystem. Consolidating the results of this study with our current understanding of how *TGF-β1* regulates microglia polarization, we highlight cell-specific metabolome reprogramming as a promising non-gene-specific therapeutic avenue for inherited retinal degenerations.

Inherited retinal degenerations

Retinitis pigmentosa (RP) is a blinding disorder that can be caused by any one of over 3100 mutations in more than 71 genes (1). In RP, many of these genes exhibit rod-specific expression and, when mutated, initiate the same biphasic rod-cone progressive pathology — a wave of rod death that drives a second, partially overlapping, wave of cone death (2). Clinically, the gradual loss of rods corresponds to symptoms such as impaired night vision and peripheral vision loss. This is followed by the secondary loss of cones, which leads to the more devastating loss of day vision (2). Unexpectedly, patients with mutations in different RP genes display first-order

decay, suggesting that the rate of vision loss is independent of the nature of the causative RP gene (3).

Limitations of current gene therapy strategy

The best hope for a cure lies in a genetic therapy that can either repair the mutation, supplement the affected gene, or both. Unfortunately, these precision medicine strategies require that the therapeutic components (such as guide RNA [gRNA] with repair template or cDNA supplement) be custom designed, engineered, tested, and FDA approved for each mutation or gene — a nearly impossible undertaking given the plethora of mutations known to contribute

to RP (3). A more widely implementable therapeutic strategy would be to target the pathways underlying secondary cone loss, which is common to many of these genetically heterogeneous forms of RP.

Cell nonautonomous degeneration of cone photoreceptors

Mutations in genes encoding for subunits of the rod-specific enzyme cyclic guanosine monophosphate (cGMP) phosphodiesterase 6 (PDE6), are responsible for approximately 72,000 cases of RP worldwide each year (4). The Cepko laboratory has pioneered the use of *Pde6* models to examine metabolic coupling and cell nonautonomous degeneration of cones in RP (5). The *PDE6β^{RDI/RDI}* mouse carries homozygous mutations in a gene encoding the β subunit of rod photoreceptor cGMP PDE6β. Rod PDE6 is a heterotrimer composed of 2 inhibitory subunits (PDE6γ) and 2 catalytic subunits (PDE6α and PDE6β) that act to hydrolyze cGMP for the closure of the cyclic nucleotide-gated (CNG) channels (4, 6). In *Pde6* mutants, rod death has been attributed to toxicity from high levels of free cGMP directly or the secondary high Ca²⁺ influx through the CNG channels when free cGMP is elevated. Elucidation of pathways responsible for cell nonautonomous degeneration of cones has emerged as a major topic of interest in light of the potential for such pathways to serve as therapeutic targets.

Precision reprogramming in imprecision medicine

In this issue of the *JCI*, Wang et al. explore the role of microglia in secondary cone degeneration by using FDA-approved adeno-associated virus (AAV) serotype 8 to deliver *TGF-β*, a major antiinflammatory cytokine that inhibits microglial activation, to the cones of 3 preclinical RP models harboring rod-specific genet-

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Authorship note: SMC and JR contributed equally to this work.

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Table 1. Cone rescue due to reprogramming of aerobic glycolysis in microglia and photoreceptors

RP model	Treatment	Target cell	Reference	Equivalent human years of useful cone vision ^A
<i>Rho</i> ^{-/-}	TGF-β1	Cones	Wang et al. (7)	~10 ^B
<i>PDE6β</i> ^{RD1/RD1}	CNTF intravitreal injection	Cones and rods	(17)	~1.4 ^B
<i>PDE6β</i> ^{RD1/RD1}	RdCVF overexpression	Cones and rods	(18)	~1.9 ^B
<i>PDE6β</i> ^{RD10/RD10} and <i>PDE6β</i> ^{RD1/RD1}	CX3CL1	Cones	(19)	~4
<i>Pde6β</i> ^{HG200/HG200}	shRNA Sirt6 ablation	Rods	(14)	~5
<i>PDE6β</i> ^{RD1/RD1}	PC upregulation	Cones	(20)	~5
<i>Pde6β</i> ^{HG200/HG200}	PKM2 ablation	Rods	(21)	~5
<i>Pde6β</i> ^{HG200/HG200}	TSC1 ablation	Rods	(15)	~5
<i>PDE6β</i> ^{RD10/RD10}	NRF2	Nonspecific (CMV promoter)	(22)	~5.77
<i>Rho</i> ^{-/-}	CNTF overexpression	Cones	(17)	~14 ^B
<i>PDE6β</i> ^{RD1/RD1}	PTEN ablation	M-opsin	(13)	~20

There is variability in the outcome measures used in the references. ^ADutta and Sengupta (16).

^BEstimate based on extrapolation from different figures within the reference. CNTF, ciliary neurotrophic factor; RdCVF, rod-derived cone viability factor; PC, pyruvate carboxylase; PKM2, pyruvate kinase isozyme M2; TSC1, tuberous sclerosis 1; NRF2, nuclear factor erythroid 2-related factor 1; PTEN, phosphatase and tensin homolog.

ic defects (7). The authors achieved sustained, cone-specific overexpression of the 3 TGF-β isoforms (TGF-β1, TGF-β2, TGF-β3) with the AAV8-human red opsin (1-3). Notably, only TGF-β1, precisely, but not TGF-β2 or TGF-β3, coding AAV8 inhibited the secondary death of cones and nearly tripled the cone number in *PDE6β*^{RD1/RD1} mice and improved visual function in *PDE6β*^{RD10/RD10} mice at 2 months of age. The anatomical rescue effects were further corroborated in a third more slowly degenerating RP mouse model, *Rho*^{-/-}. However, when the researchers depleted all M1 and M2 microglia in untreated *PDE6β*^{RD1/RD1} mice, cone survival failed to improve. Conversely, microglia depletion nullified AAV8-TGF-β1-mediated cone rescue, indicating the importance of precision reprogramming microglia in facilitating TGF-β1 therapeutic efficacy.

The neuroprotective contributions of microglia can be understood in terms of their metabolic tuning in many degenerative disorders (8). The resting metabolic state of MO homeostatic microglia is oxidative phosphorylation. However, under stress, a shift from oxidative phosphorylation to aerobic glycolysis induces the M1 microglia phenotype through activation of the TAM (TYRO3, AXL, and MERTK) receptor tyrosine kinase signaling path-

way (9). This metabolic switch is associated with the chronic release of neurotoxic inflammatory factors, which may underlie tissue damage and photoreceptor death. More recently, TGF-β1 has been found to precisely suppress glycolytic M1 programming while promoting oxidative M2 microglia polarization (10, 11). Interestingly, M2 polarization is known to promote neuroprotection and antiinflammatory response, which is abrogated with diminished TGF-β1. Altogether, these findings suggest that secondary cone loss is mediated by a pathological upregulation of glycolytic processes in M1 microglia, which can be countered by reestablishing M2 oxidative metabolism. Wang et al. demonstrate that AAV-mediated supplementation of TGF-β1 can counteract some aspects of neurodegenerative disorders in preclinical *Pde6b* and *Rho* models, thereby suggesting the therapeutic potential of precisely reprogramming glycolytic M1 to oxidative M2 microglia (7).

Future directions in precision reprogramming

Cell type-specific metabolic reprogramming can rejuvenate aerobic glycolytic balance within the eye and rescue vision. Importantly, Wang and colleagues elegantly validate the therapeutic feasibility

of using AAVs (7). AAVs have become the most widely used vector in FDA trials due to their low immunogenicity and cytotoxicity, and the ability of their serotype to be engineered for cell type-specific targeting (1). As such, they represent the ideal gene delivery vehicle to mediate metabolomic reprogramming in microglia as well as other retinal cells. As proposed by the Hurley hypothesis, different retinal cells are metabolically coupled (12), and thus how these dynamics are disrupted in dystrophic retinas may pose another critical direction for cone preservation (Table 1).

Recently, the photoreceptors and retinal pigmented epithelial cells have been identified as other cell types that suffer from dysregulation of glycolytic and oxidative phase-shifting during RP progression. In healthy retinas, rods take up glucose from the retinal pigmented epithelium (RPE) and convert it to lactate via aerobic glycolysis, even in the presence of oxygen. Much like microglia, young RPE prefers an oxidative metabolic state and uses the generated lactate as a substrate for oxidative phosphorylation and a suppressor for RPE-specific glucose consumption. In contrast, in *PDE6* dystrophies, the lactate production decreases as rods die, stagnating NAD⁺ consumption in the RPE. The resultant accumulation of NAD⁺ stimulates glycolysis in the RPE, which depletes all the available glucose from the choriocapillaris as an energy source. As the delicate mutualism between oxidative and glycolytic tissue processes fails, cones begin to die from malnutrition (5). These findings suggest that boosting aerobic glycolysis in dystrophic photoreceptors might counter photoreceptor death from RP-induced metabolic imbalance. Clinicians can apply the AAV vector delivery implemented by Wang et al. to drive diseased photoreceptors toward enhancing aerobic glycolysis (Table 1). Genetically ablating inhibitors of aerobic glycolysis, either *Tsc1* (13) or *Sirt6* (14), increase the number of photoreceptors in RP retinas (13-15). SIRT6 is a master promoter of aerobic glycolysis via the rate-limiting enzymes for glycolysis and GLUT1-mediated glucose transport. Future studies directed toward precision metabolome reprogramming may substantially advance the development of a gene agnostic therapy for neurodegenerative disorders.

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