Pten controls the timing of retinal progenitor cell differentiation in part through its regulation of glycolytic flux

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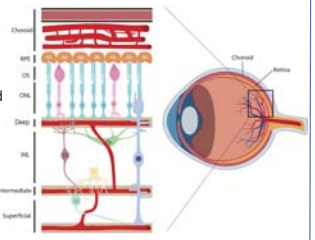
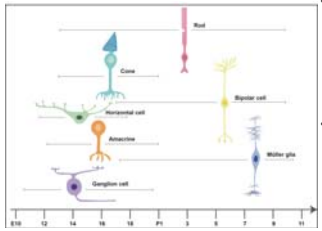
Introduction

- Visual impairment is devastating, affecting 2 billion individuals worldwide
- A notable cause of vision loss in several blinding eye diseases, such as age-related macular degeneration, is the death of of photoreceptors [1]
- Designing novel therapies requires a deep understanding of the factors affecting photoreceptor differentiation and maturation to form functional circuitry



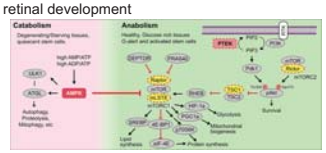


- Seven retinal cell types are generated from a multipotent pool of **retinal progenitor cells (RPCs)** in a defined order during development.
- Cell fate decisions by individual RPCs are controlled by lineage-specifying transcription factors, but the choice to differentiate at any given time is stochastic

- It is poorly understood how environmental signals and their downstream signal transduction cascades influence RPC fate selection

Purpose

- To study the effect of signal transduction cascades on RPC fate selection and differentiation during retinal development
  - We focused on Pten, a phosphatase that functions as a negative regulator of PI3K, mTORC1 and other signal transduction cascades



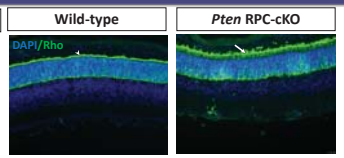
Materials and Methods

- We investigated the role of *Pten* in retinal development using an RPC-specific conditional knock-out (cKO) approach.
- We crossed a *Pax6::Cre* driver and *Pten*^{fl} allele to generate *Pten RPC-cKO* mice [4, 5]

Timing of rod differentiation is disrupted in *Pten* RPC-cKOs

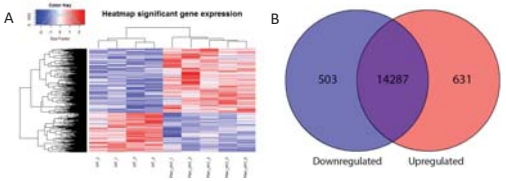
- Birthdating experiments were performed at E12.5, revealing that more RPCs give rise to rod photoreceptors in Pten RPC-cKOs

Early Maturation of Rod Outer Segments



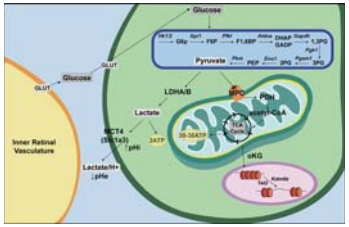
- Pten RPC-cKOs show early maturation of rod photoreceptor outer segments (arrows) compared to wildtype controls, in which rod outer segments had not yet formed at P7 (arrowhead)

Transcriptomic differences in P0 *Pten* RPC-cKOs



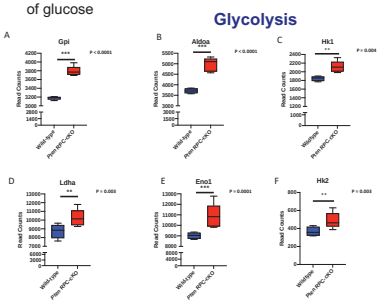
- Bulk RNA-seq of P0 wild-type and Pten RPC cKOs
- Heatmap (A) showing expression of individual genes in both groups. Venn diagram (B) showing a total of 1075 dysregulated genes in *Pten* cKO retinas. Blue circle = downregulated genes, Red circle = upregulated genes, Purple = genes not differentially expressed.

Upregulation of glycolysis genes in *Pten* RPC-cKO



- There are two main bioenergetic pathways – glycolysis, which occurs in the cytosol, and oxidative phosphorylation (OXPHOS), which occurs in the mitochondria.

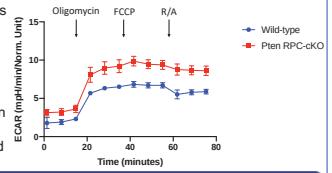
- glucose is broken down to pyruvate via glycolysis, a multi-enzymatic cascade that does not require O_2 and which generates 2 ATP per molecule of glucose



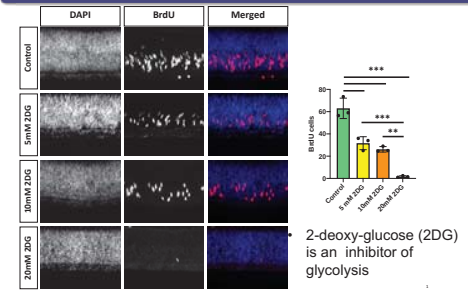
- A large number of glycolytic genes are upregulated in P0 *Pten* RPC-cKOs

Functional Increase in glycolysis in Pten RPC-cKO

- P0 *Pten* RPC-cKO RPCs showed increase in ECAR, a measure of delta pH that is an indirect indicator of increased intracellular pH, and suggestive of an increase in glycolysis, which leads to increased lactate

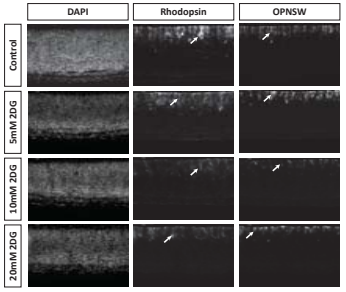


Inhibition of glycolysis blocks RPC proliferation



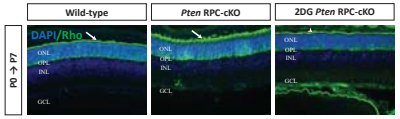
- 2-deoxy-glucose (2DG) is an inhibitor of glycolysis

Glycolysis inhibition decreases photoreceptor marker expression



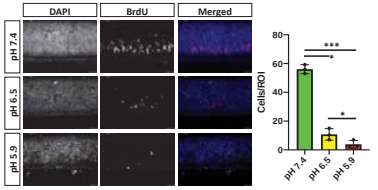
- Treating P0 wildtype explants with the glycolytic inhibitor 2DG in vivo (30 mg/kg) decreased rod photoreceptor marker (rhodopsin) and cone photoreceptor marker (OPNSW) expression

Inhibition of glycolysis reversed outer-segment prematurity in *Pten* RPC-cKO



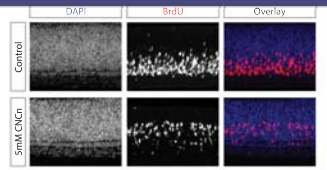
- Treating Pten RPC-cKO daily with the glycolytic inhibitor 2DG (30 mg/kg) from P0-P7 delayed the maturation of rod outer-segments (arrowhead)

Lowering pHi phenocopies 2DG effects



- In anaerobic conditions, pyruvate is converted to lactate and exported out of the cell along with a H^+ ion by lactate/H^+ symporters (MCT1/4), with a resultant decrease in extracellular pH (pHe) and increase in intracellular pH (pHi)
- Growing retinal explants in different pH resulted in reduction of retinal proliferation with reduced pH levels

Blocking lactate transport inhibit RPC proliferation



- Growing retinal explants in α-cyano-4-hydroxycinnamic acid (CNCn), a MCT1/4 lactate transporter blocker, resulted in reduction of retinal proliferation

Conclusions

- *Pten RPC-cKO* mice showed early rod photoreceptor differentiation and early outer-segment maturation at P7
- Glycolytic pathway genes are up in P0 *Pten* RPC-cKOs and glycolysis was functionally increased
- Inhibition of glycolysis reduced proliferation and photoreceptor marker expression in wildtype animals
- Inhibition of glycolysis in *Pten* RPC-cKO mice delayed the maturation of photoreceptor outer-segment
- Lowering pHi that mimic reduction in glycolysis also resulted in reduced retinal proliferation, same in blocking lactate transporters
- These finds will aid our understanding in developing novel therapies to replace degenerated photoreceptors

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