Identifying Protocadherin Gamma-Dependent Signaling Pathways that Regulate Interneuron Survival in the Retina

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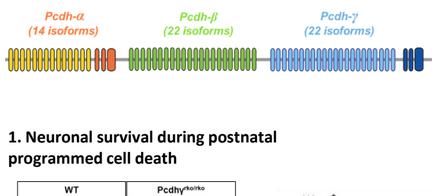
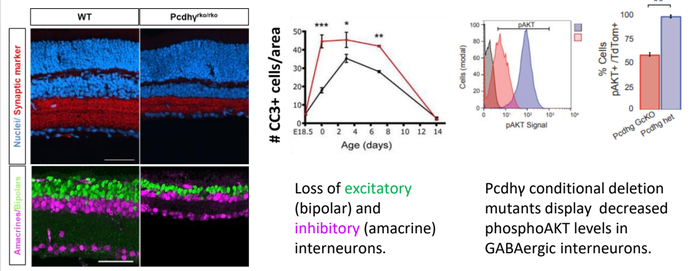
Introduction

The clustered protocadherins (Pcdhs) encode a large family of transmembrane proteins. The 58 Pcdh genes are tandemly-arrayed in three clusters; Pcdh-alpha, Pcdh-beta, and Pcdh-gamma. In particular, the gamma Pcdhs (Pcdhγ) are especially important for promoting neuronal survival .



1. Neuronal survival during postnatal programmed cell death



Loss of excitatory (bipolar) and inhibitory (amacrine) interneurons.

2. PhosphoAKT expression

Pcdhγ conditional deletion mutants display decreased phosphoAKT levels in GABAergic interneurons.

Lefebvre et al., 2008; Chen et al., 2012 — Carriere et al., 2020

Goal

To determine if Pcdhγ promotes GABAergic interneuron survival through the PI3K-AKT pathway in the mouse retina

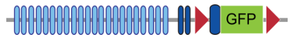
Objectives

1. Determine the function of PTEN in post-mitotic GABAergic interneuron survival in the retina.
2. Test for genetic interactions between PTEN and Pcdhγ

Methods

Conditional deletion alleles

Inactivates the 22 *Pcdhγ* genes

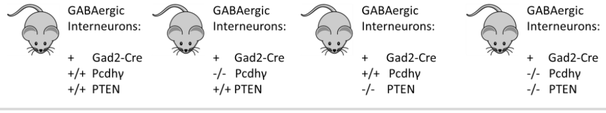


Inactivates *PTEN* gene

X

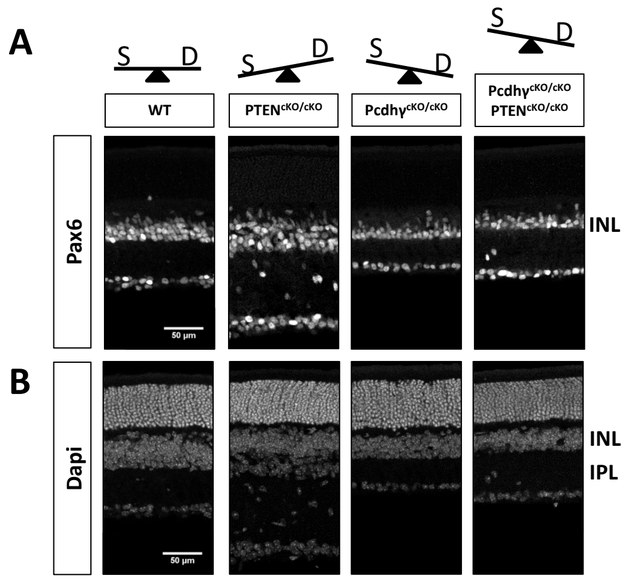
Target GABAergic Amacrine cells with Cre driver

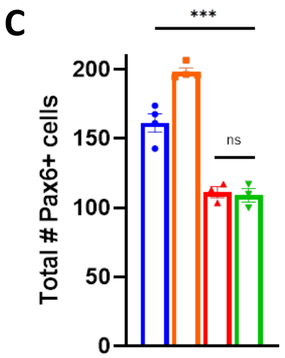
Gad-Cre: Early post-mitotic targeting of GABAergic neurons



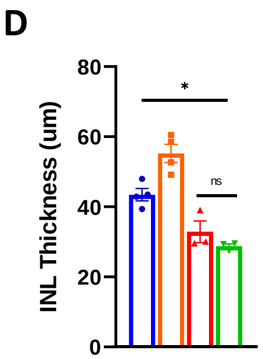
Results

1. Conditional deletion of PTEN is not sufficient to rescue GABAergic interneuron survival in Pcdhγ mutants

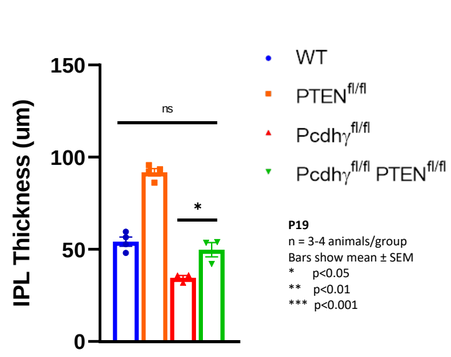




Quantification of Pax6+ amacrine cells. (A, C) PTEN mutants have an increased number of amacrine cells, while Pcdhγ mutants have reduced numbers compared to WT. Amacrine cell number is not rescued in Pcdhγ;PTEN double mutants. This suggests that Pcdhγ may function downstream of PTEN.

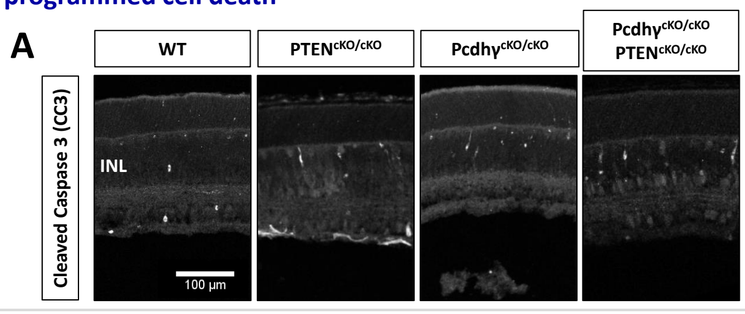


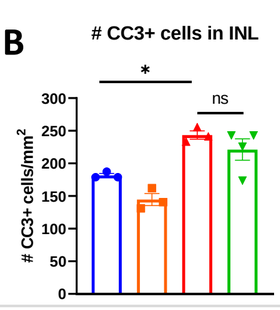
Quantification of inner nuclear layer thickness. (B, D) PTEN mutants have an increased thickness of the INL, while Pcdhγ mutants have reduced thickness compared to WT. INL thickness is not rescued in Pcdhγ;PTEN double mutants. These results are reflected in the quantification of amacrine numbers.



Quantification of inner plexiform layer thickness. (B, E) PTEN mutants have an increased thickness of the IPL, while Pcdhγ mutants have reduced thickness compared to WT. IPL thickness is rescued in Pcdhγ;PTEN double mutants. This suggests increased neurite outgrowth (ongoing experiment).

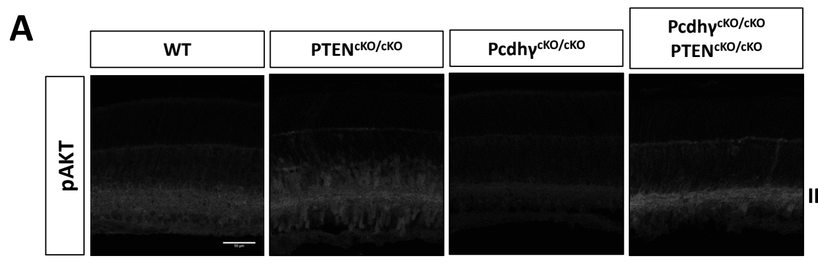
2. Conditional deletion of PTEN does not reduce apoptosis in Pcdhγ mutants during developmental programmed cell death

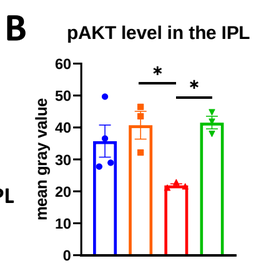




Quantification of CC3+ cells in the P7 INL. Pcdhγ mutants have an increased number of apoptotic CC3+ cells in the INL compared to WT. The number of apoptotic cells is not rescued in Pcdhγ;PTEN double mutants, as double mutants display similar numbers compared to the Pcdhγ mutants. 3-4 animals/group. Bars show mean ± SEM. * p<0.05, ** p<0.01, *** p<0.001.

3. Conditional deletion of PTEN elevates phosphoAKT expression in Pcdhγ mutants despite increased programmed cell death





Quantification of pAKT expression in the P7 IPL. Pcdhγ mutants have decreased levels of pAKT in the IPL compared to WT. pAKT levels are restored to WT levels in Pcdhγ;PTEN double mutants. This suggests that elevated pAKT alone is not sufficient to prevent excess apoptosis. 3 animals/group. Bars show mean ± SEM. *p<0.05, ** p<0.01, *** p<0.001.

Conclusions

1. PTEN regulates retinal GABAergic interneuron survival and in a cell-type autonomous manner.
2. Pcdhγ and PTEN converge in a similar pathway to regulate GABAergic interneuron survival.
3. Pcdhγ may function downstream of PTEN in the PI3K-AKT pro-survival pathway.

Future Work

Identify the regulatory relationship between PTEN and Pcdhγ

1. Are protein levels of Pcdhγ affected in PTEN mutants vs. WT?
2. Does PTEN regulate the cell surface expression of Pcdhγs?
3. Does Pcdhγ phosphorylation level change in PTEN mutants vs. WT?