Pten Regulates the Stereotyped Morphology of Starburst Amacrine Cells

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Abstract

Starburst amacrine cells (SACs) have a highly stereotyped dendritic architecture which is critical for their computation in the direction-selective circuit. SACs laminate in the S2 and S4 sublaminae of the IPL by extending a dendritic process which expands to form their planar-radially symmetric arbor. To achieve their characteristic morphology across all members of their subtypes, SACs must carefully regulate the growth and refinement of their dendritic arbors during development. Phosphatases and protein homing (Pten) serve as the primary negative regulator in the P32-Akt pathway, which is critical for the growth and survival of developing cells. In neurons, Pten is particularly important for the regulation of dendrites and axons to form synaptic connections during development. Previous work has shown that peripheral deletion of Pten via targeting of retinal progenitor cells leads to widespread disruptions in the cell density, migration, and lamination of multiple neuronal subtypes. In order to assess the role of Pten specifically in regulating retinal development, we use a ChAT-Cre driver line to delete Pten selectively in SACs, beginning at postnatal day 1 (P1). Preliminary results indicate that SACs lacking Pten exhibit normal migration and mosaic spacing, but their dendrites show hypertrophic branching and disrupted lamination in the IPL. Ultimately, this work will elucidate how Pten functions in the development of neurons with highly stereotyped dendritic arbors.

Schematic of the P32-Akt-mTOR Pathway

Cell density, mosaic spacing and stratification remain grossly normal in Pten<cKO> SACs

In contrast to peripheral deletion of Pten, loss of Pten specifically from SACs at P1 does not affect early developmental processes. A, B: Representative flatmounts taken from Pten+ and Pten<cKO> SACs. B: Quantification reveals no changes in SACs within the IPL and GCL. E, F: SACs in Pten<cKO> retain abnormal somal shape and stray dendritic projections, particularly between the INL and GCL. G: Quantification with JPL/mosaic shows that overall SAC stratification in S2/S4 the IPL remains grossly unchanged in Pten<cKO> retinas. Scissors = 25um.

Validation of Pten Deletion

Perforated Laminar Reconstruction

ChAT-Cre; Pten<cKO>

ChAT-Cre; Pten<+/+>

C

Pten<cKO>

ChAT-Cre; Pten<+/+>

ChAT-Cre; Pten<cKO>

Pten<cKO>

ChAT-Cre; Pten<+/+>

In vivo labeling reveals aberrant morphology in Pten<cKO> SACs

Cells were sparsely labeled with AAV-FLuc-TDT/Tomat2 injections at P2, and then imaged at P21. Both images are from SACs in the INL. A, B: Pten<cKO> SACs show normal, canonical SAC morphology. B: Pten<cKO> SACs show a clear increase in branching and dendritic density, as well as increased dendritic crossing. Scissors = 30um.

References:

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Future Directions

• Perform single cell morphology analysis at other time points (P7, P14, P21)
• Further investigate the regulation of the P32 Pathway in SACs (Tdt+/−)
• Investigate changes in synaptic density (P52-95-labeling)
• Extend analysis to other amacrine cells (GCL-AC)

Sholl Analysis reveals increased density in proximal dendrites

A. Example image describing how Sholl analysis is performed. Concentric circles are drawn from the soma, and the number of intersections with dendritic processes are quantified for each circle. B. ChAT analysis shows an increase of dendritic density in the first 75% of the arbor for Pten<cKO> SACs in both the GCL and INL. This corresponds to the SAC dendritic compartment which receives input from upstream bipolar cells.