Characterizing the cellular phenotype of AAV-mediated Pten knockout in the peripheral nervous system

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Introduction

- Pten (phosphatase and tensin homolog) acts as a molecular brake on axon growth after nerve injury.
- Altering cell-specific expression of the Pten gene may thus provide a means for targeted enhancement of the inherent regenerative potential of the PNS.
- Pten knockout in mouse sciatic nerve injury models has been shown to increase outgrowth of the distal axon and speed recovery; however, little is known regarding the effects of Pten knockout on the soma of adult PNS neurons.
- Here, we assessed the effects of Pten knockout on DRG neuronal soma size *in vivo* using an Adeno-Associated Virus (AAV)-mediated gene editing strategy.

Methods

- We performed bilateral mouse sciatic nerve injections of AAV carrying either Cre recombinase or RFP into Pten$^{flox/flox}$ Tdtomato$^{flox/flox}$ transgenic mice, resulting in DRGs with sparsely infected RFP+ Pten KO neurons or control RFP+ neurons.
- Mice were sacrificed at 2 weeks post-injection and the DRGs fixed, processed with IHC, and imaged to measure the cell sizes across four different DRG subtypes based on immunolabeling (NF200 for large light neurons, CGRP for peptidergic neurons, TrkB for LTMRs, parvalbumin for proprioceptive neurons).

Results

- We found that AAV-mediated knockout of Pten increases soma size in primary sensory neurons of the PNS.
- Larger neuron morphology was seen in Pten KO versus WT neurons in all four DRG subtypes (large light neurons: p=0.0212, peptidergic: p=0.0328, LTMR: p=0.0004, proprioceptive: p<0.0001).

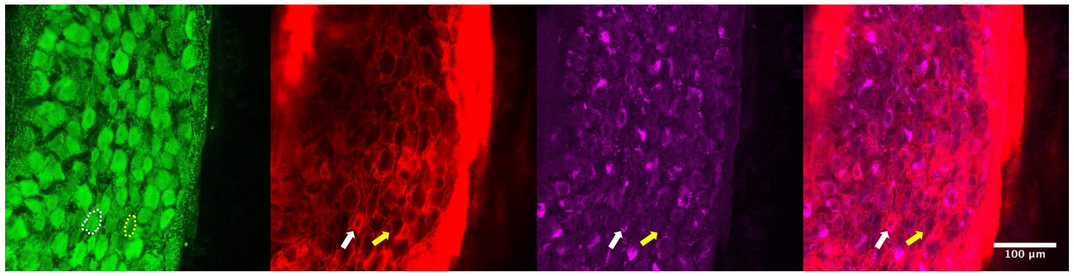


Figure 1. Immunohistochemistry of L4 DRG from Pten$^{flox/flox}$ Tdtomato$^{flox/flox}$ mouse after intrasciatic injection of AAvrg hysn-CRE. Antibodies used are MAP2 (neurons/green), RFP (Pten/red), and CGRP (peptidergic/purple). White outline and arrow indicates a Pten KO peptidergic neuron. Yellow outline and arrow indicates a Pten WT peptidergic neuron.

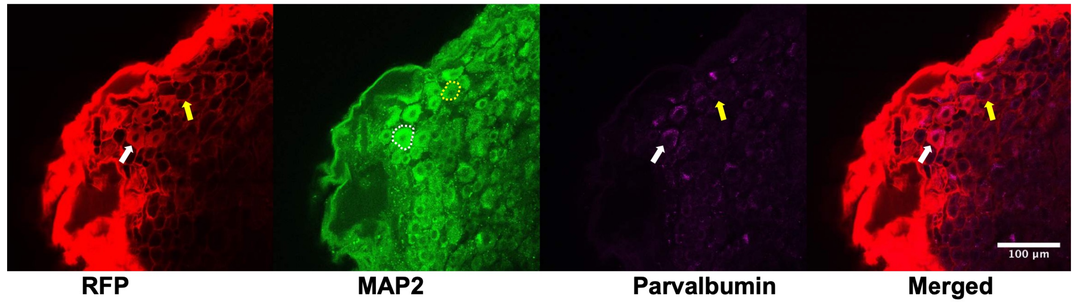


Figure 2. Immunohistochemistry of L1 DRG from Pten$^{flox/flox}$ Tdtomato$^{flox/flox}$ mouse after intrasciatic injection of AAvrg hysn-CRE. Antibodies used are RFP (Pten/red), MAP2 (neurons/green), and parvalbumin (proprioceptive/purple). White outline and arrow indicates a Pten KO proprioceptive neuron. Yellow outline and arrow indicates a Pten WT proprioceptive neuron.

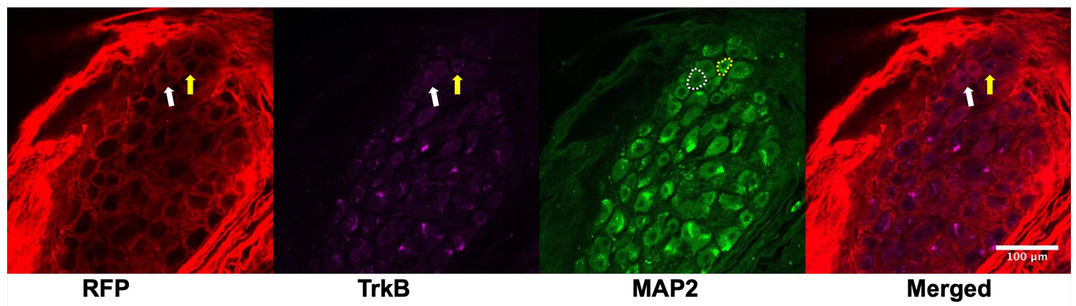
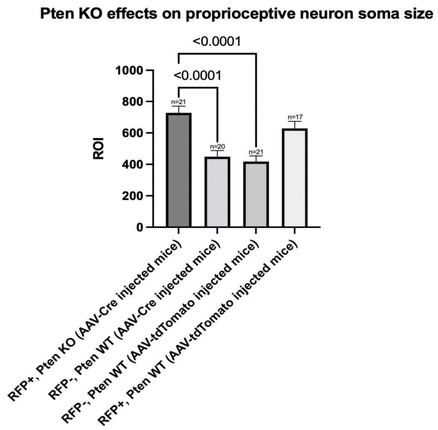
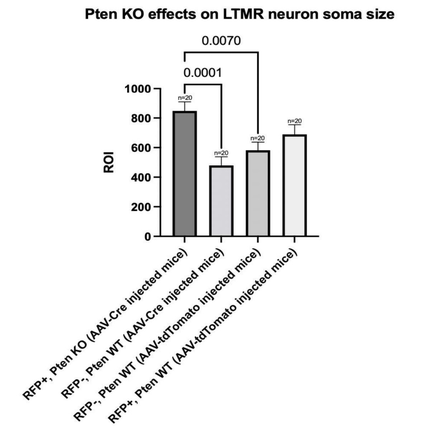


Figure 3. Immunohistochemistry of L1 DRG from Pten$^{flox/flox}$ Tdtomato$^{flox/flox}$ mouse after intrasciatic injection of AAvrg hysn-CRE. Antibodies used are RFP (Pten/red), TrkB (LTMR/purple), and MAP2 (neurons/green). White outline and arrow indicates a Pten KO LTMR neuron. Yellow outline and arrow indicates a Pten WT LTMR neuron.





Conclusions

- AAV-mediated Pten knockout in the PNS results in functional hypertrophy of neuronal soma.
- This characterization furthers our understanding of the underlying cell biology of enhanced axon regeneration after loss of Pten.

Acknowledgements

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