A new therapeutic gene for RGC survival and axon regeneration in optic neuropathies

Mishal Rao[1], Chia-Chun Liu[1], Kun-Che Chang[1]
[1]Department of Ophthalmology, University of Pittsburgh School of Medicine
Supported by Glaucoma Research Shaffer Grant

INTRODUCTION

- In optic neuropathies, axons of retinal ganglion cells (RGCs) are injured and do not regenerate, which leads to progressive RGC loss and neurodegeneration.
- No effective treatment exists to restore vision loss in optic neuropathies.
- There is a growing interest in identifying new therapeutic targets and strategies to promote RGC regeneration and functional recovery.
- TPPP3 gene encodes a protein called tubulin polymerization-promoting protein family member 3, which is also known as p20.
- In previous studies, Tppp3 has been identified in the RGC cluster and has been shown to play a role in axon regeneration.
- In this study, we provide new insights into the potential of Tppp3 as a therapeutic molecule for promoting RGC survival and axon regeneration.

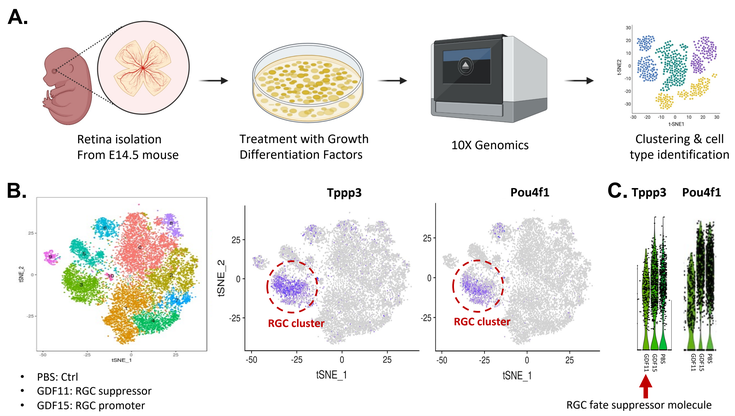
METHODS

- Primary RGCs isolated from P3 mice pups by immunopanning were used to study neurite outgrowth.
- Primary RGCs were transduced with AAV2-Tppp3 overexpression or AAV2-control and AAV2-Tppp3 shRNA or AAV2-scramble for three days. Primary RGCs were immunostained with Tuj-1, a neuronal marker, to visualize neurite outgrowth and quantified using ImageJ.
- For *in-vivo* studies, 8-10 week-old mice were treated with AAV2-Tppp3 overexpression or AAV2-control by intravitreal injection in OS two weeks before optic nerve crush (ONC). Animals were sacrificed for RGC survival and axon regeneration studies two weeks after ONC. Flatmount retinas were stained with RBPMS for RGC counting. Regenerative axons were visualized by CTB-555.
- RNA sequencing was performed on mice retinas collected two days after ONC.
- The study was conducted in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and approved by IACUC at the University of Pittsburgh. All experiments were performed in at least independent triplicate, analyzed by Student's t-test or one-way ANOVA, and considered significant if $P < 0.05$.

RESULTS

Figure 1: **Identification of novel RGC marker by single cell RNA sequencing analysis.**

(A) GDF-treated samples were analyzed by single cell RNA sequencing. **(B)** RGC marker (Pou4f1) and Tppp3 showed overlap and co-expression in the RGC cluster. **(C)** Tppp3 expression was reduced by GDF11 treatment, an RGC suppressor.



RESULTS

Figure 2: **Tppp3 expresses in mouse RGCs in vitro and in vivo.**

Several RGC marker genes were probed in adult mouse RGC **(A)** and optic nerve **(B). (B)** Tppp3 is degraded in the crushed optic nerve. **(C)** Tppp3 was detected in the ganglion cell layer. ~75% of Brn3a+ cells express Tppp3 and arrows indicate the Brn3a co-labelled cells. **(D)** Immunostaining data revealed that Tppp3 was mainly expressed in the soma of P2 primary RGCs, which was confirmed by co-labeling with Tuj-1, a neuronal marker.

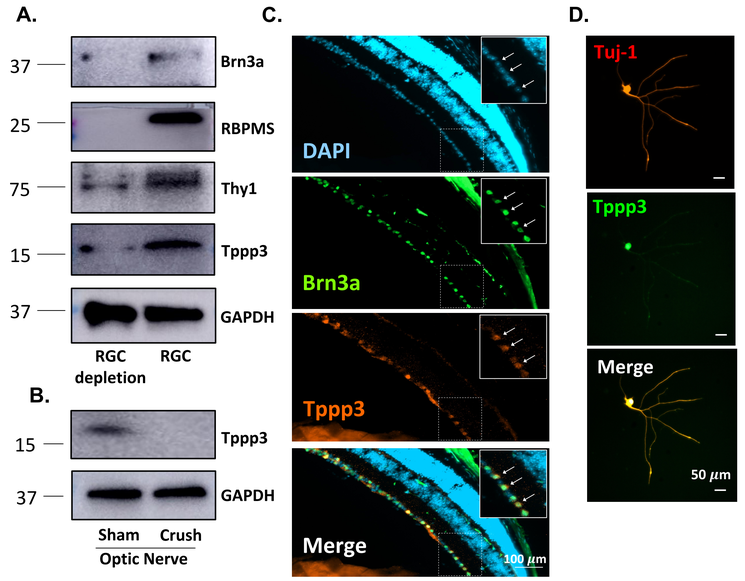
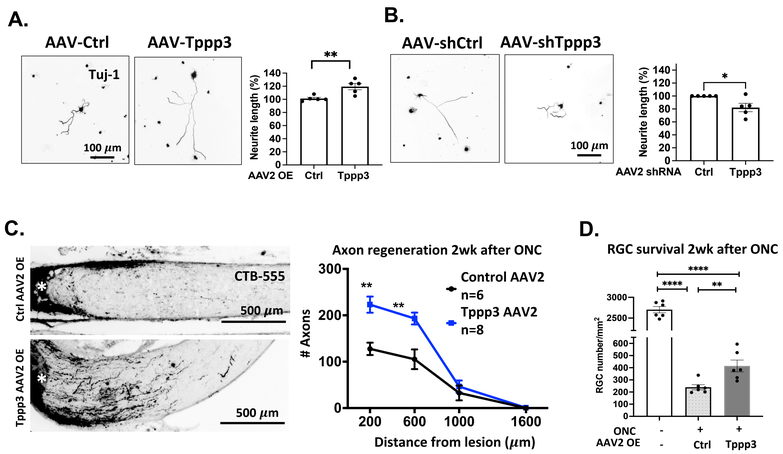


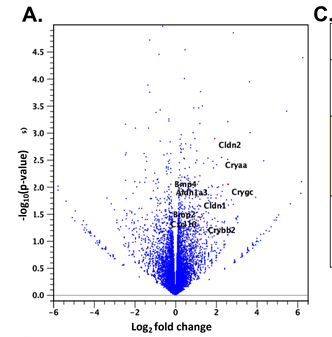
Figure 3: **Functions of Tppp3 in RGCs *ex-vivo* and *in-vivo***

(A) Overexpression of Tppp3 increases RGC neurite outgrowth by ~20%. **(B)** Knockdown of Tppp3 reduced neurite outgrowth by ~20%. In *vivo*, overexpression of Tppp3 promotes axon regeneration **(C)** and RGC survival **(D)** 2 weeks after optic nerve crush (ONC).



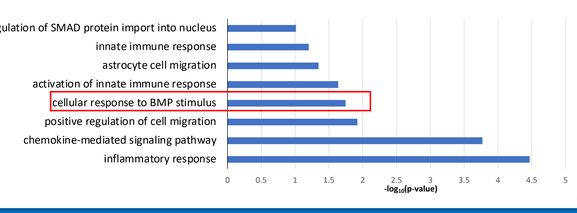
RESULTS

Figure 4: **Tppp3 overexpression increases BMP4 signaling and inflammation-related genes. (A)** Several genes were upregulated after Tppp3 AAV2 OE in the whole retina 2d after ONC. **(B)** The table highlights genes related to axon regeneration and survival. **(B)** Inflammation-related and BMP signaling pathway GO terms are highlighted.



C.

Gene	Function
Aldh1a3	Associated with RA signaling, LOF results in aberrant **eye development**
Bmp4	Activation of the BMP4/smad1 pathway promotes **retinal ganglion cell survival** and **axon regeneration**
Cryaa, Crybb2, Crygc, and Crygs	Crystallins play a role in **ocular neuroprotection** and **axonal regeneration**



CONCLUSIONS

- Tppp3 is expressed in RGCs and is found across all RGC sub-types
- Tppp3 promotes neurite outgrowth, axon regeneration, and RGC survival
- Tppp3 overexpression increases inflammation-related genes and BMP4.
- Tppp3 acts as a regulator to stimulate the intrinsic regenerative ability of RGCs, which would provide a translational impact on regenerative medicine.

NEXT STEPS

- Determine the specific mechanism by which TPPP3 overexpression induces inflammation-related genes, as well as whether the BMP signaling pathway is involved in this process.
- Examine the role of Tppp3 on RGC function and vision restoration after optic nerve injury by conducting pattern electroretinography.

ACKNOWLEDGEMENTS

The work was supported by the NIH Core Grant P30-EY008098 (KCC), Eye and Ear Foundation of Pittsburgh (KCC), an unrestricted grant from the Research to Prevent Blindness, New York, NY (KCC) and **Shaffer Grant from the Glaucoma Research Foundation** (KCC).

NIH National Eye Institute · Supported By Research to Prevent Blindness · GLAUCOMA RESEARCH FOUNDATION · The Eye & Ear Foundation of Pittsburgh