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Tep1 Regulates Yki Activity in Neural Stem Cells in Drosophila Glioma Model

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Tep1 regulates Yki activity in Neural Stem Cells in *Drosophila* Glioma model

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Figure 1: Introduction to the Model system

In Drosophila, the larval CNS is comprised of two brain lobes and a ventral nerve cord (Figure 1A). There are two types of progenitor or neural stem cells (NSCs aka neuroblasts) in the Drosophila larval CNS – the optic lobe (OL) neuroepithelium and the central brain (CB) neuroblasts (Figure 1A'). In the CB and ventral nerve cord (VNC) the neuroblasts of Type I and Type II lineages are found. During brain development, NSCs undergo self-renewing asymmetric cell divisions to produce a neuroblast and a smaller daughter cell, the ganglion mother cell (GMC) that divides once more to differentiate into neuron or glia. To ensure that the correct number and type of neurons are made, NSCs must coordinate cell cycle entry and exit with a strict developmental timing. Deregulation of these NSCs could give rise to overgrowth of the brain associated with the formation of tumors.

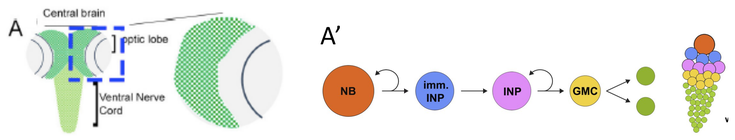

Figure 1- A: Cartoon showing larval CNS of Drosophila with Central Brain (CB) marked in green checkered boxes as ROI. A': Cartoon showing asymmetric cell divisions in a NB cell to give rise to INP (blue and purple), a GMC (yellow) and glial cells (Green).

Hippo/MST Pathway and Tep/CD109

- The Hippo pathway is known to regulate the proliferation of two cell populations in the larval Drosophila brain: glia and the neuroepithelium.
- The pathway restricts neuroblast proliferation potential and neuronal cell number to regulate brain size during the development of the Drosophila CNS.
- Deregulation of Hippo signaling in neuroblasts causes a substantial increase in overall brain size. Recently, CD109/ (Tep1 in Drosophila) was shown to be a novel upstream regulator of YAP/TAZ during glioma growth (Minata et al., 2019).
- CD109 belongs to the group of Thioester containing proteins (TEPs) which are involved in antimicrobial response in both vertebrate and invertebrate models and raise inflammatory responses in vertebrates.
- CD109 is a cancer stem cell marker with a preferential enrichment of CD109+ tumor cells in the perivascular region in high-grade gliomas. MES recurrence is associated with an increase in CD109 expression and CD109 is also shown to cause resistance to radiotherapy with poor clinical outcomes in GBM patients.

Figure 2: Downregulation of Tep1 Reduces Proliferation in Glioma

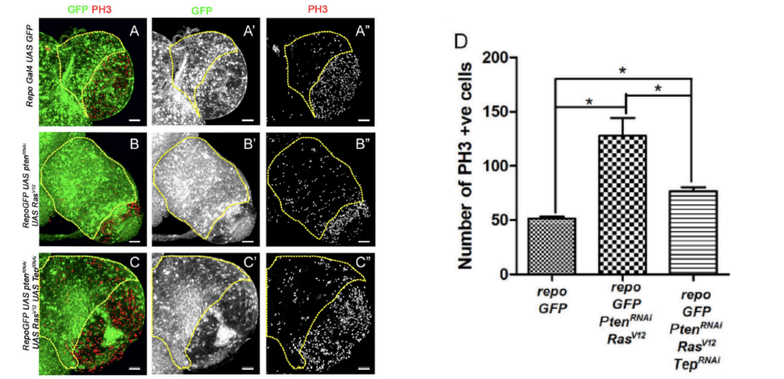

Figure 2: Comparisons of PH3 positive cell numbers in the central brain region of Drosophila third instar larval brain (see, schematic in Fig. 1A) are shown. Glial cells are marked by GFP (green, gray) and PH3 (red, gray). (D) Quantification of number of PH3 positive cells in the central brain is shown in the graph.

Unpaired 2-tailed T-test with n = 5, 95% confidence was performed using GraphPad Prism 5, $p < 0.05$. Yellow ROI boxes indicate area in which PH3 positive cells are counted. All images were scanned at identical magnification [20, scale bars, 100 mm = 62px].

Figure 3: Glioma grow by Specification of Ectopic Neuroblasts

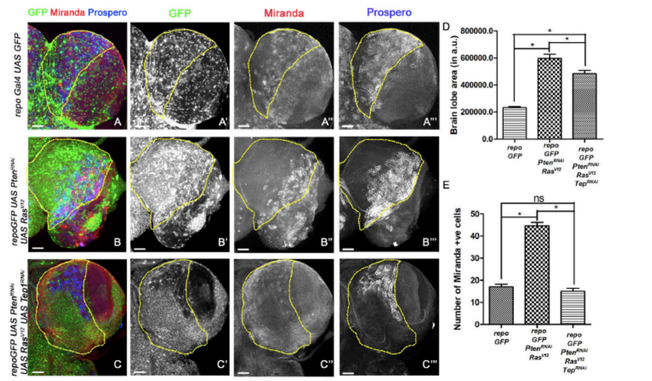

Figure 3: Loss of Tep1 suppresses ectopic Neuroblasts that drive glioma growth. Panels show comparisons of Miranda (red) and Prospero (blue) positive cells in the central brain of *Drosophila* larval CNS from third instar larvae

(D) Quantification of brain lobe size was done using the magnetic lasso tool in Photoshop to create an ROI for measuring pixel values. **(E)** Quantification of number of Mira positive cells in the central brain is shown in the graph.

For **(D)** and **(E)** unpaired 2-tailed *T*-test with $n = 5$, 95% confidence was performed using GraphPad Prism 5, $p < 0.05$. Yellow ROI boxes indicate area in which Mira +ve cells are counted. All images were scanned at identical magnification [20 ×, scale bars, 100 µm = 62px].

Figure 4: Downregulation of Yki Results in Decreased Glioma Growth Due Decreased Neuroblast Proliferation

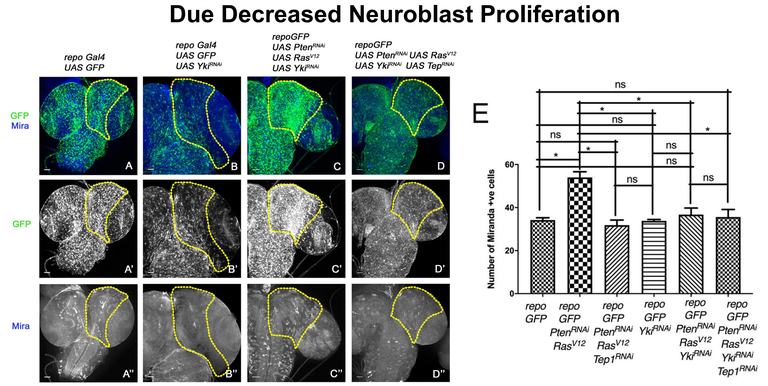

Figure 4: Downregulation of Yki reduces glioma growth and neuroblast number in larval CNS. **(A–D'')** panels show a comparison of neuroblast number in the central brain region (the yellow line marks the ROI in one brain lobe for each genotype). In all panel's glia are marked by GFP (green, gray), and neuroblasts by Mira expression (blue, gray).

(E) Quantification of number of Mira positive cells in the central brain region is shown for the indicated genotypes. Unpaired 2-tailed *T*-test with $n = 5$, 95% confidence was performed using GraphPad Prism 8, $p < 0.05$. Yellow ROI boxes indicate area in which Mira positive cells are counted. All images were scanned at identical magnification [20 ×, scale bars, 100 µm = 62px].

Conclusions and Future Directions

- The coactivation of PI3K and MAPK/EGFR pathways promotes the formation and progression of glioma causing the brain lobes to appear elongated and misshapen ultimately causing lethal neoplasms.
- We identified Yki modifier, Tep1 (Drosophila ortholog of CD109) is a modifier of glioma phenotype due to its effect on NSC number.
- the Tep-Yki interaction plays an important role in glioma growth due to the effect of Tep1 on Yki-mediated neuroblast proliferation. The reduction in the neuroblast number may be attributed to the effects of Tep1 downregulation, which compromises Yki mediated stem cell function in glioma thereby identifying a new upstream regulator of Yki in the larval CNS.
- The exact mechanisms by which Tep1 interacts with Yki remains to be defined.
- The similarity of glioma reduction phenotypes of downregulation of Tep1 and downregulation of Yki or both suggests that Tep1 may act through Yki and the Hippo pathway to regulate NSC number, or that Tep1and Yki synergistically regulate NSC number in Drosophila larval brain.

Acknowledgments

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