Selective elimination and analysis of nestin (+) optic nerve laminar region-neural progenitor cells (ONLR-NPCs)

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Purpose

Selectively eliminating Nestin(+)/Sox2(+) ONLR NPCs is difficult to achieve because: 1) Most current approaches to eliminate Sox2(+) cells results in depletion of these cells in both the retina and ONLR (Fig 1B-C). 2) Most Nestin(+) transgenic animals do not express the nestin-associated transgene in the ONLR (Fig 1A). 3) It is difficult to determine whether effective treatments are reaching the target cells. We wanted to identify an appropriate model and approach to selectively deplete ONLR-NPCs, using either transgenic mice expressing nestin-promotor drivers or knockin Sox2-Cre mice (Bernstein et al, PNAS, 2020).

Methods

Nestin(+) ONLR cells were targeted by three methods: C57BL/6-Tg(Nes-cre/ERT2)KEisc/J X Rosa26 LoxP(DTA); Sox2-cre/ERT2 knockin X Rosa26 LoxP(DTA) were evaluated for ONLR-nestin elimination after retrobulbar Tamoxifen injection. Alternatively, transgenic C57BL/6-Tg(Nes-TK*-EGFP)145 Sker mice, constitutively express GFP and viral thymidine kinase (TK) (Kernie, 2012) and GFP(+) cells in the ONLR and retina of these mice can be evaluated directly using GFP immunohistochemistry. We eliminated Nestin(+) cells in the latter transgenic strain using either IP injection X 3 weeks of systemic Ganciclovir (50mg/kg/d) or by 3 week CNS adminstration of intrathecal ganciclovir (GCV: Cytovene, 47.5mg/ml) using 2002/2004 Alzet pumps attached to a intraventricular canaliculus (Alzet 3). We evaluated ganciclovir distribution into the ONH and retina by doping the GCV fill with 0.5% fluorescein, analyzing retinal and optic nerve fluorescence using a Heidelberg SD-OCT instrument with the blue peak fluorescent setting.

Results

The Nestin-Cre-ER2 transgenic showed no loss of either ONH-nestin or Sox2 nuclei. There was also no change in the number of axons or myelination, suggesting that nestin-expressing ONLR-NPCs do not express the transgene. In contrast, Sox2-Cre knockin animals showed a loss of both ONLR Sox2(+) nuclei and nestin expression, coupled with increased loss of anterior ON myelination. However, the loss of axons could be due to loss of Sox2 (+) retinal amacrine cells and/or oligodendrocyte precursor cells (OPCs) as well as in ONLR-NPCs. This clouds interpretations.

To further define effects of isolated ONLR-NPC loss, we turned to a newer, widely used transgenic model, the Nestin-Tk (GFP) mouse, which expresses GFP in every cell that also expresses the *delta* (viral) thymidine kinase (TK), which renders cells susceptible to ganciclovir (GCV). We then evaluated the nestin-Tk(GFP) model, and found that it expresses GFP in a subset of ONLR-NPCs, as well as a subset of retinal Mueller cells, and CNS-Hippocampus, but not in distal ON (Fig 2). One week post-implantation of a 2004-4 week Alzet pump into the lateral ventricle loaded with GCV and 0.5% fluorescein.

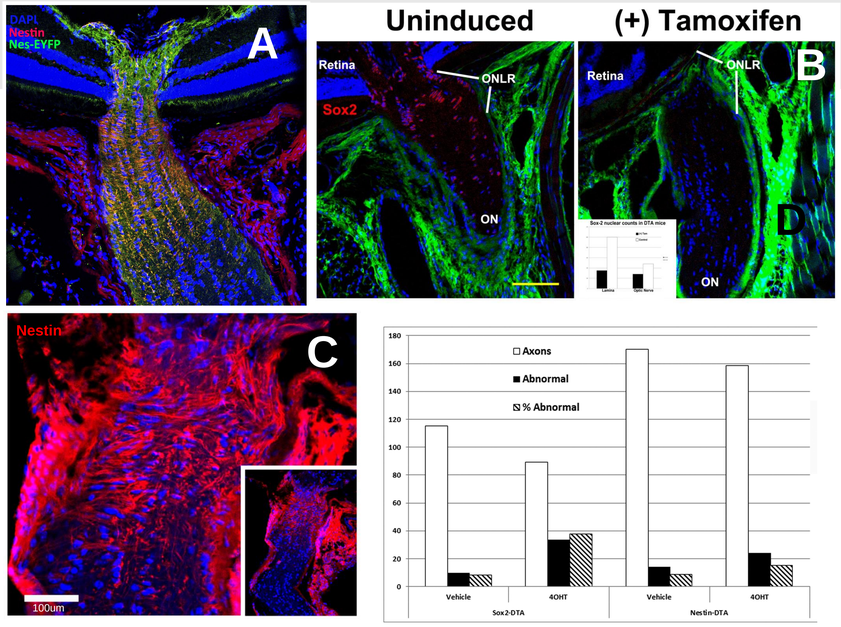

Figure 1. Histology of nestin and Sox2 expression in transgenic/knockout mice A: No loss of Nestin-driven YFP expression in transgenic Nestin-Cre X DTA mice. 2 weeks post-Tamoxifen injection, there is no loss of nestin or YFP positivity in the ONLR. B. Comparison of Sox2 (+) nuclei in Sox2-Cre X DTA knockin mice. In the vehicle treated nerve, ONLR nestin expression (in red) is strong. Two weeks post-Tamoxifen, there ONLR-nestin depletion (inset) D. TEM-Axonal analysis in the two strains. There was no significant change in ON axon number or myelination in the Eisch transgenic model, with or without Tamoxifen. In contrast, the Sox2-Cre knockin had fewer axons overall, and tamoxifen administration resulted in further axonal loss and significant loss of myelinated axons.

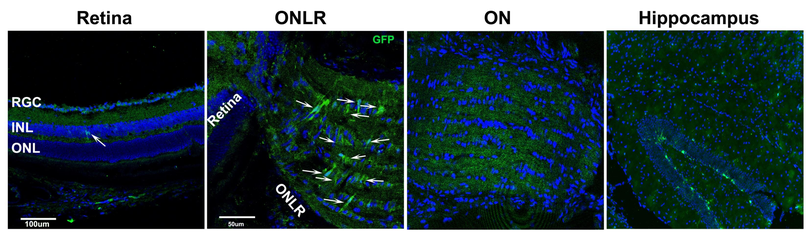

Figure 2: GFP expression in the Nestin-Tk-(GFP) transgenic (60d) mouse. Sparse GFP(+) cells in the retina correspond to nestin (+) Mueller cells. There is strong GFP expression in the ONLR and Hippocampus. No nestin signal is detectable in the distal ON.

we imaged the retina and ON using the bluepeak feature in the Heidelberg SD-OCT instrument (Heidelberg, Germany). Only the ONH fluoresced (Fig 3B). We used this approach to selectively eliminate ONLR-NPCs without significantly impacting the retina (Fig 3C).

Figure 3: Selective elimination of Nestin-Tk-(GFP) cells using an intrathecal Alzet pump. A. Bluepeak evaluation of animal without fluorescein-loaded Alzet pump. The retina and ONH are dark. B. Bluepeak evaluation of Alzet pump loaded with GCV and 0.5% fluorescein. A detectable signal is seen in the ONH, but not in the retina. C. Loss of GFP expression in the ONLR 4 weeks post-implantation.

Conclusions

Transgenic nestin promotor strains have widely different expression patterns which must be examined at the ONLR to determine suitability.

Knockin mice strains, rather than transgenic strains, with their positional effects, are more likely to be effective in selective elimination of ONLR-NPCs.

Combining Intrathecal Alzet pumps with a fluorescent dye can be used for positive identification of isolated ONH treatments, using the Heidelberg instrument.

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