The Role of Ascl1 in NG2 Cells in the Spinal Cord

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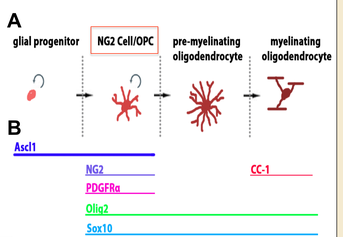
INTRODUCTION

NG2 cells, one of the major glial cell populations within the central nervous system, are highly proliferative cells identified by the expression of the NG2 proteoglycan. NG2 cells can be maintained in a proliferative state indefinitely, or differentiate into oligodendrocytes (Figure 1) [3]. A recent study showed that deletion of the NF1 and p53 tumor suppressor genes specifically within NG2 cells produced brain tumors in a mouse model, indicating that NG2 cells may be a cell of origin for gliomas [2].

Ascl1, a proneural basic-helix-loop-helix (bHLH) transcription factor that is highly expressed in neural progenitor cells, is also expressed in NG2 cells in the embryonic and adult spinal cord (Figure 2). Ascl1 is up-regulated in gliomas and other cancers, including small cell lung carcinoma, where knockdown of Ascl1 inhibits tumor growth [1]. Therefore, understanding Ascl1's role in NG2 cells is important in that Ascl1 represents a potential therapeutic target in gliomas. Although prior studies have shown that loss of function of Ascl1 affects NG2 cell specification and differentiation, the specific role of Ascl1 in NG2 cells during embryonic and postnatal development remains unknown [4,5].

Figure 1. Development of NG2 cells in the CNS. (A) NG2 cells can proliferate or differentiate. (B) Ascl1, NG2 and PDGFRa are expressed in NG2 cells. Olig2 and Sox10 are expressed throughout oligodendrocyte development. CC-1 is a mature oligodendrocyte marker.



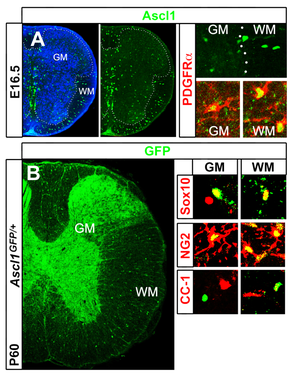


Figure 2. Ascl1 expression in NG2 cells. (A) Ascl1 staining in grey matter (GM) and white matter (WM) of embryonic spinal cord at E16.5. (B) Ascl1 (GFP) staining in GM and WM of adult spinal cord at P60.

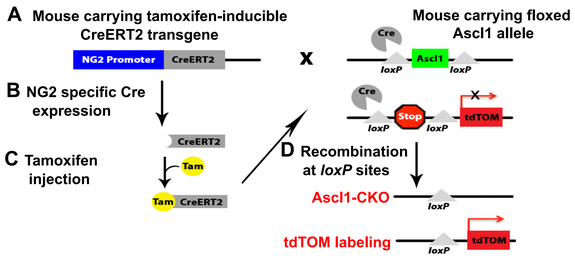
HYPOTHESIS

Ascl1 is required to maintain NG2 cells in a proliferative state, and the loss of Ascl1 in NG2 cells will alter their development and differentiation.

MATERIALS AND METHODS

We conditionally knocked out Ascl1 specifically within NG2 cells (Ascl1-CKO) in the embryonic or adult spinal cord using a NG2-CreERT2;tdTOM mouse strain in which the tdTomato fluorescence reporter (tdTOM) was incorporated to allow visualization of NG2-expressing cells that had Cre recombinase activity (Figure 3).

Figure 3. Conditional deletion of Ascl1 using *NG2CreERT2* mice.



MATERIALS AND METHODS

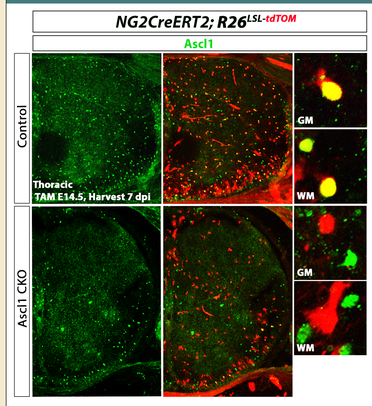
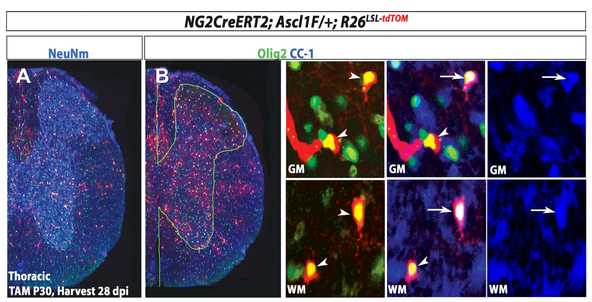


Figure 4. Ascl1 is CKO in NG2 Cells. Ascl1 (green) is efficiently knocked out in NG2-CreERT2 expressing cells (red) in both gray and white matter regions of the spinal cord.

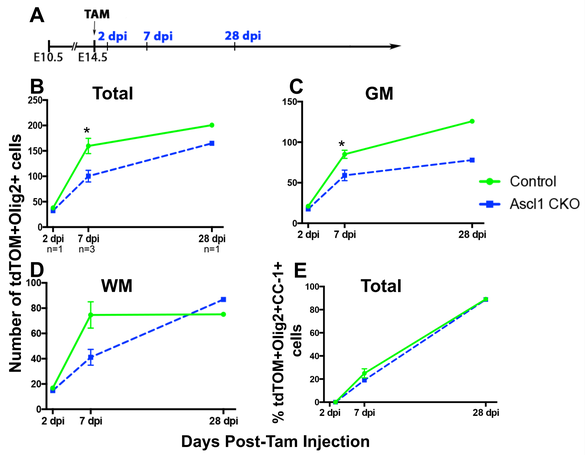
We assessed whether Ascl1 was required for controlling cell number and differentiation by counting the number of tdTOM-labeled cells and determining the percentage of these cells that express mature oligodendrocyte markers (Figure 5).

Figure 5. Quantification. (A) NeuN staining used to identify GM/WM boundaries. (B) Olig2+tdTOM+ cells (arrowheads) were quantified and the percentage of these cells expressing CC-1 (arrows) was determined.



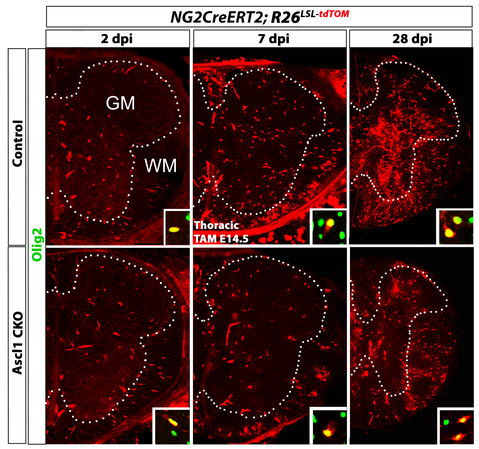
RESULTS

Figure 6a. Embryonic Ascl1-CKO differentially alters the number of NG2 cells in GM and WM of the spinal cord. (A) Tamoxifen was injected at E14.5 and spinal cords were analyzed at 2, 7, and 28 days post-tamoxifen injection (dpi). (B, C) Ascl1-CKO reduces total and GM Olig2+tdTOM+ cells at 7 and 28 dpi. (D) In the WM, there is an increased number of Olig2+tdTOM+ cells in the Ascl1 CKO at 28 dpi. (E) Embryonic Ascl1-CKO does not alter NG2 cell differentiation.



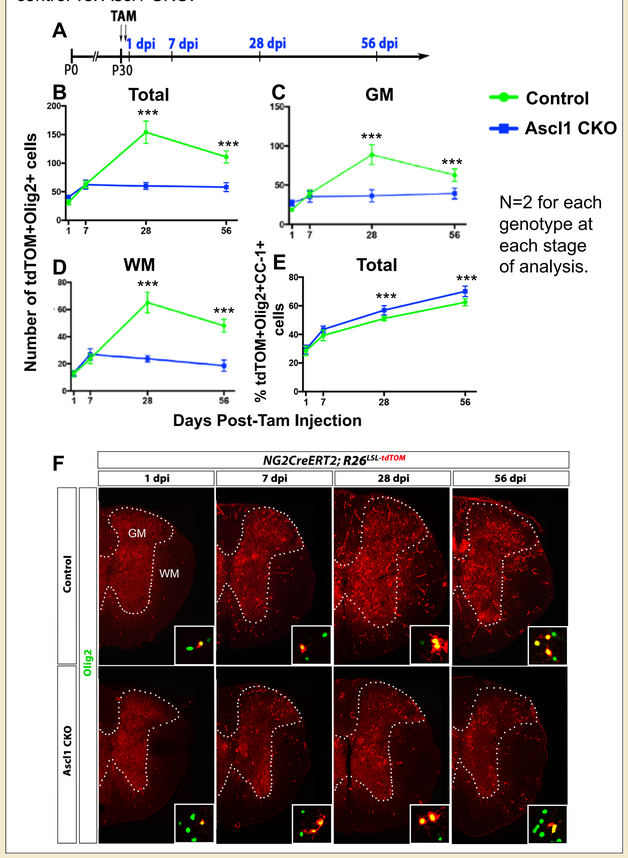
RESULTS

Figure 6b. Representative images showing tdTOM+ cells in control vs. Ascl1 CKO. Quantification shown in 6a.



RESULTS

Figure 7. Adult Ascl1-CKO decreases the number of GM and WM NG2 cells and increases their differentiation in the spinal cord. (A) Tamoxifen was injected at P30 and the spinal cords were analyzed at 1, 7, 28, and 56 dpi. (B,C,D) The number of Olig2+tdTOM+cells is reduced in the Ascl1-CKO at 28 and 56 dpi in both the GM and WM. (E) The percentage of CC-1+ Olig2+tdTOM+ cells is slightly increased in the Ascl1-CKO at 28 and 56 dpi. (F) Representative images showing tdTOM+ cells in control vs. Ascl1 CKO.

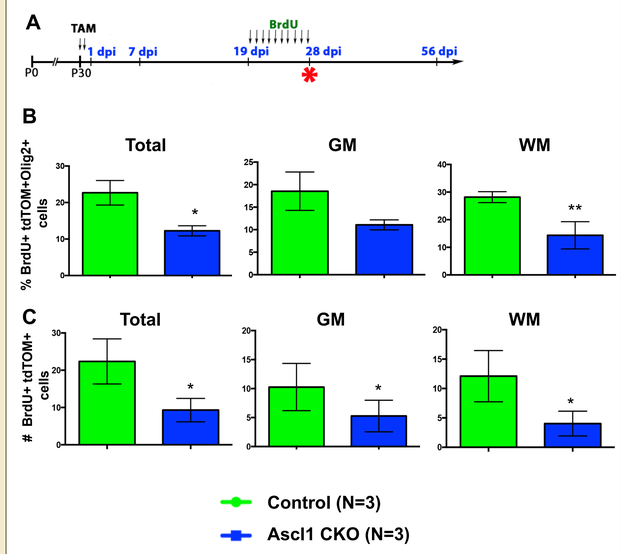


N=2 for each genotype at each stage of analysis.

RESULTS

To investigate if the change in cell number in the adult Ascl-CKO is due to altered proliferation, we used bromodeoxyuridine (BrdU), a thymidine analog that is incorporated into newly synthesized DNA, to specifically label replicating cells. The number of BrdU-labeled cells was then compared between the control and Ascl-CKO (Figure 8).

Figure 8. Adult Ascl1-CKO reduces NG2 cell proliferation in the GM and WM. (A) BrdU was injected for 10 days in adult Ascl1-CKO and control mice. Spinal cords were analyzed at 28 dpi. The percentage (B) and number (C) of BrdU+ Olig2+tdTOM+cells is reduced in the Ascl1-CKO in both the GM and WM.



CONCLUSION

Embryonic Ascl1-CKO led to a reduction in the number of NG2 lineage cells in the GM, but an increase in the number of NG2 lineage cells in the WM. These results indicate that during embryonic spinal cord development:

a. Ascl1 is required to regulate NG2 cell proliferation and/or cell survival

b. Ascl1 may play a different regulatory role in the GM vs. WM

Adult Ascl1-CKO resulted in a significant reduction in the number of NG2 lineage cells in both the GM and WM. The reduction in the number of BrdU-labeled NG2 lineage cells seen in the Ascl1-CKO demonstrates that this is due, at least in part, to a reduction in NG2 cell proliferation. Unlike embryonic Ascl1-CKO, adult Ascl1-CKO slightly accelerates NG2 cell differentiation. These results indicate that during adult spinal cord development:

a. Ascl1 is required to regulate NG2 cell proliferation

b. Ascl1 may also play a role in NG2 cell differentiation

REFERENCES

[1] Jiang T et al. (2009). Cancer Res 69: 845–854.
[2] Liu, C. et al. (2011). Cell 146, 209-221.
[3] Nishiyama, A. et al. (2009). Nat Rev Neurosci 10, 9-22.
[4] Sugimori, M et al (2007). Development 134, 1617-1629.
[5] Sugimori, M. et al. (2008). Development 135, 1271-1281.

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