Roles of Ascl1 and Olig2 in the Transcriptional Regulation of Astrocytogenesis

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Introduction

There are two classes of morphologically distinct astrocytes (AS): the "protoplasmic" astrocytes of the gray matter (GM), and the "fibrous" astrocytes of the white matter (WM) (Figure 1) [1]. It is not known how these two classes of astrocytes are generated from glial progenitor cells during development.

Ascl1 and Olig2 are basic helix-loop-helix (bHLH) transcription factors highly expressed in certain neural progenitor cells, and are known to be involved in neurogenesis and oligodendrogenesis throughout the CNS; however, their role in astrocytogenesis is less well explored.

Ascl1:

Recent evidence shows that Ascl1+ progenitor cells give rise to astrocytes in the spinal cord (SC) [2]. It remains to be seen whether the same is true in the brain.

It was also found that Ascl1-lineage AS clones in the spinal cord (one clone representing all the progeny of a single AS-progenitor cell) are spatially restricted to either the GM or WM, but not both (Figure 1) [2], but that conditional knockout of Ascl1 in these cells produces bipotent progenitor cells capable of producing both GM protoplasmic and WM fibrous astrocytes [2], suggesting the possibility that an earlier (Ascl1-negative) GM/WM bipotent astrocyte progenitor cell may exist.

Olig2:

It was previously thought that Olig2 was not expressed in the astrocytes of the spinal cord, and even that Olig2 expression may inhibit astrocytogenesis [3]; however more recent evidence shows that Olig2 is required for astrocytogenesis in the WM (but not GM) of the brain and spinal cord, and that Olig2 is in fact expressed by the immature GFAP+ AS themselves in the neonatal/perinatal brain [4]. Whether this expression pattern is also observed in the spinal cord remains unresolved.

We consider the following questions:

(1) Do Ascl1+ progenitors give rise to astrocytes in the brain, as was seen in the spinal cord?
(2) Do astrocyte clones, in general, display the GM/WM spatial restriction observed in Ascl1-lineage astrocytes?
(3a) Is Olig2 expressed by GFAP+ astrocytes in the spinal cord, as was observed in the brain?
(3b) If it is, is this Olig2 expression required for astrocytogenesis in the spinal cord?

Hypotheses:

(1) Yes, Ascl1+ cells will give rise to AS in the brain as seen in the SC.
(2) Yes, results will recapitulate those seen for Ascl1-lineage cells.
(3a) Yes, AS of the SC will express Olig2, as in the brain, with WM-AS expressing Olig2 more often than GM-AS (reflecting their developmental requirement for Olig2).
(3b) Yes, inducible conditional knockout of Olig2 in an AS-specific, time-specific manner will affect development of both GM and WM-AS in the neonatal SC.

Figure 1. Ascl1 in glial development in the spinal cord. (A) GFAP and ApoE are markers specific to the astrocyte lineage, while Sox10 and CC1 are specific to the oligodendrocyte lineage. (B) Ascl1-lineage GM-protoplasmic (red) and WM-fibrous (purple) astrocytes are derived from separate Ascl1+ progenitors. (C) Examples of spatially restricted clones, each deriving from a separate Ascl1+ progenitor. (D) Examples of GM-protoplasmic (left) and WM-fibrous (right) astrocytes by fluorescence microscopy. [1,2]

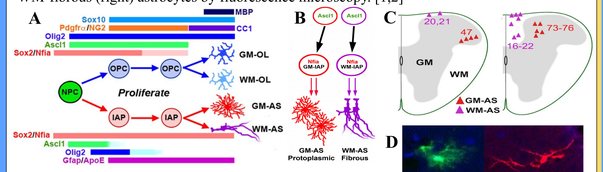
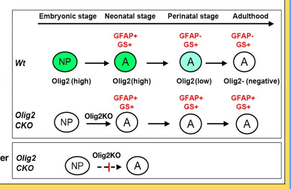


Figure 2. Olig2 in AS development in the CNS. (A) Olig2 is expressed by the immature GFAP+ AS in the neonatal brain. (B) Constitutive knockout of Olig2 (Olig2KO) prevents development of WM-AS (but not GM-AS). [4]
Note that the constitutive nature of the Olig2KO introduces potential confounds that cannot be controlled for. The current study instead utilizes the inducible CreERT2 system for time- and cell-specific conditional knockout of Olig2.



Methods and Results:

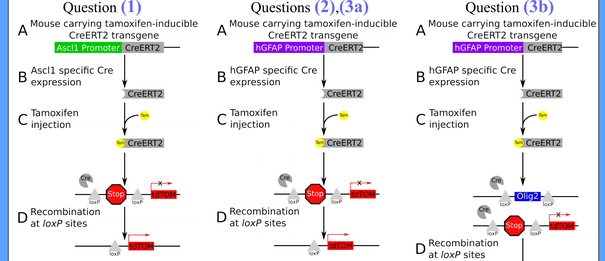
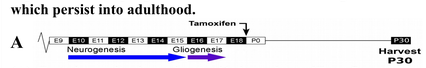


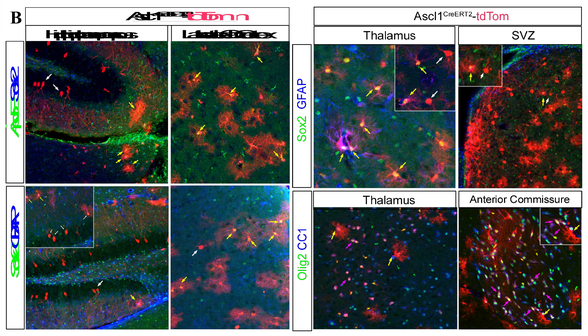
Figure 3. CreERT2;tdTom genetic system.
(1) Tamoxifen-inducible CreERT2 under the Ascl1 promotor was used to label Ascl1+ progenitor cells in the brain. All cells expressing Ascl1 at the time of induction (and all their progeny) were subsequently permanently identifiable by their tdTom fluorescence.
(2,3a,3b) Substituting the hGFAP promotor provided inducible Cre recombinase expression specifically in astrocyte-lineage cells. In (2), this was used to drive Confetti recombination in astrocyte-lineage cells. In (3a), this was used to simply label astrocyte lineage cells with tdTom fluorescence. In (3b), this was used in mice whose Olig2 genes had been "floxed" (as shown), allowing inducible deletion of Olig2 specifically within astrocyte-lineage cells, producing a time- and cell-type-specific Olig2 conditional knockout (Olig2-CKO).

Methods and Results:

Figure 4. Ascl1+ progenitors in the neonatal brain give rise to astrocyte cell populations which persist into adulthood.

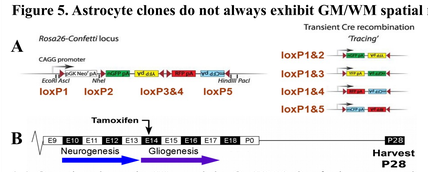


(A) Experimental schematic: Ascl1-dependent tdTom fluorescence was induced in Ascl1+ cells during the neonatal period of development. Adult brains were harvested 30days post-induction and were immunohistochemically labeled for factors specific to mature astrocytes (ApoE, GFAP) and oligodendrocytes (CC1).



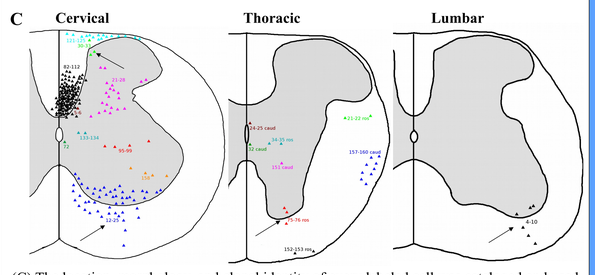
(B) Ascl1-lineage cells (red) were identified by their tdTom fluorescence. tdTom+;ApoE+ and tdTom+;GFAP+ double-positive astrocytes (yellow arrows) derived from neonatal Ascl1+ progenitors were observed in every major cortical and subcortical structure, showing that neonatal Ascl1+ progenitors do give rise to astrocytes throughout the brain, and that these astrocyte cell populations persist into adulthood
Ascl1-lineage oligodendrocytes (purple arrows) and neurons (white arrows) were also present.

Figure 5. Astrocyte clones do not always exhibit GM/WM spatial restriction.

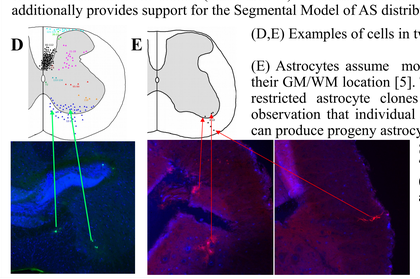


(A) Genetic schematic: We used the CreERT2-Confetti system under the promoter for hGFAP (an AS-specific marker) to give sparse labeling of astrocytes in multiple colors, so that any clone (one clone representing all the progeny of a single AS-progenitor cell) will be far from and visually distinct from other clones, allowing us to trace single AS-progenitor clones through development into adulthood.

(B) Experimental schematic: hGFAP-dependent Cre recombination was induced during embryonic gliogenesis. Adult spinal cords were obtained, sectioned, and analyzed by fluorescence microscopy.



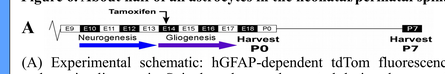
(C) The location, morphology, and clonal identity of every labeled cell was cataloged and used to construct a clonal map of AS distribution in the spinal cord, revealing the presence of non-GM/WM-restricted AS clones (black arrows). It should also be noted that this clonal map additionally provides support for the Segmental Model of AS distribution in the spinal cord.



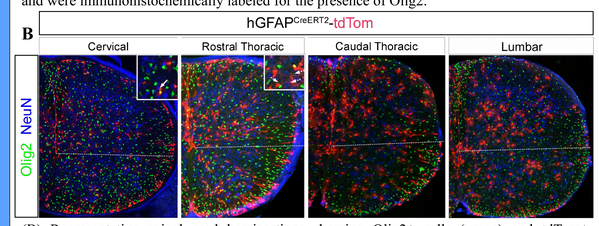
(D,E) Examples of cells in two non-restricted clones.

(E) Astrocytes assume morphologies as dictated by their GM/WM location [5]. Thus the presence of non-restricted astrocyte clones involves the additional observation that individual astrocyte progenitor cells can produce progeny astrocytes of both morphologies. Seen here (in red) are GM "protoplasmic" astrocytes (left) and a WM "fibrous" astrocyte (right).

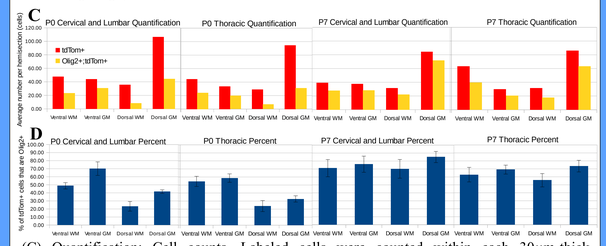
Figure 6. About half of all astrocytes in the neonatal/perinatal spinal cord express Olig2.



(A) Experimental schematic: hGFAP-dependent tdTom fluorescence was induced during embryonic gliogenesis. Spinal cords were harvested during the neonatal and perinatal periods and were immunohistochemically labeled for the presence of Olig2.

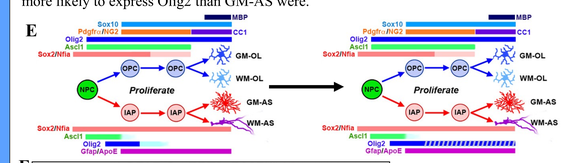


(B) Representative spinal cord hemisections showing Olig2+ cells (green) and tdTom+ astrocytes (red). The presence of Olig2+;tdTom+ double-positive cells (i.e. astrocytes expressing Olig2) was quantified.

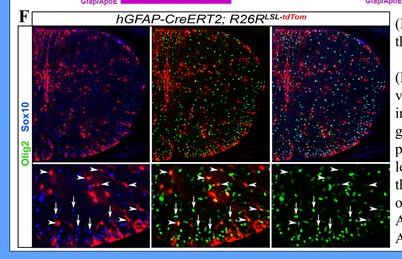


(C) Quantification: Cell counts. Labeled cells were counted within each 30µm-thick hemisection, with counts separated by spinal cord level, developmental period, and ventral/dorsal and GM/WM location.

(D) Olig2 is in fact expressed in about half of the immature GFAP+ astrocytes of the neonatal/perinatal spinal cord, with slightly higher expression rates in the perinatal period than at birth. Importantly, despite their known Olig2 developmental requirement, WM-AS were not more likely to express Olig2 than GM-AS were.



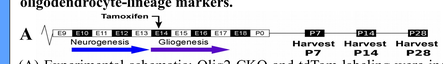
(E) Updating Figure 1a to reflect the new data.



(F) Olig2 expression levels were visibly lower in astrocytes than in oligodendrocytes within any given hemisection (bottom right panel). Lower Olig2 expression levels in AS may explain why this expression has been overlooked in the past.
Arrowhead = astrocyte
Arrow = oligodendrocyte

To determine whether this newly discovered Olig2 expression in these cells is required for astrocytogenesis in the spinal cord, we performed an inducible time-specific and astrocyte-lineage-specific (GFAP-dependent) conditional knockout of Olig2, both heterozygous (F/+) and homozygous (F/F).

Figure 7. Heterozygous Olig2 conditional deletion produces AS-lineage cells expressing oligodendrocyte-lineage markers.



(A) Experimental schematic: Olig2-CKO and tdTom labeling were induced during embryonic gliogenesis specifically in hGFAP+ (AS-lineage) cells. Spinal cords were harvested 12 days post-induction (P7). Additional data will be collected from spinal cords harvested at P14 and P28.

(B) Heterozygous conditional deletion of Olig2 produced tdTom+ cells (cells derived from AS-lineage GFAP+ precursors) expressing oligodendrocyte-lineage-specific markers (such as Sox10), in addition to astrocyte-lineage-specific markers (such as GFAP).

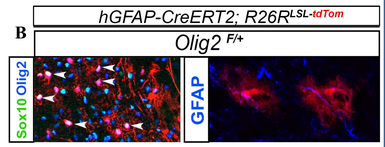
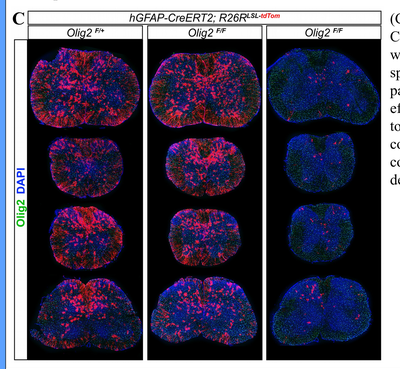


Figure 7 (cont.). Homozygous Olig2-CKO may (or may not) ablate both WM and GM AS development or maintenance in the neonatal SC.



(C) The homozygous Olig2-CKO produced mixed results, with each Olig2-CKO (F/F) spinal cord showing one of two patterns: either a) no noticeable effect on AS number compared to control, or b) an almost-complete lack of labeled AS consistent with ablation of AS development or maintenance.

Conclusions

Ascl1+ progenitors in astrocytogenesis in the brain

Astrocytes, oligodendrocytes, and neurons derived from Ascl1+ progenitors were observed throughout the brain. These results build upon previous findings that Ascl1+ progenitors produce neurons and oligodendrocytes in the embryonic brain, adding the discovery that they also produce astrocyte populations throughout the brain which persist into adulthood.

Clonal mapping and GM/WM spatial restriction of AS clones

Astrocyte clones do not always exhibit the GM/WM spatial restriction observed in Ascl1-lineage astrocyte clones. Non-restricted astrocyte clones were observed at cervical, thoracic, and lumbar levels, and in both the dorsal and ventral regions of the spinal cord. Presence of naturally occuring non-restricted astrocyte clones constitutes strong evidence for the existence of a GM/WM bipotent astrocyte progenitor cell. Further work isolating and analyzing these cells is suggested.

These results are consistent with previous findings that Ascl1 determines the ratio of GM astrocytes to WM astrocytes in the SC [2], and together with previous findings in Ascl1-CKO mice suggesting that non-restricted astrocyte clones undergo additional cell divisions (compared to spatially restricted clones)[2], these results suggest a possible mechanism for this activity: Ascl1 may control the ratio of GM to WM astrocytes by guiding the spatial segregation of astrocyte clones of differing proliferative potentials into either the GM or WM. Future studies involving BrdU and Ascl1-CKO in hGFAPCreERT2-Confetti mice will be instrumental to testing this proposed mechanism.

Olig2 expression in GFAP+ AS of the neonatal/perinatal spinal cord

Olig2 is in fact expressed in about half of the immature GFAP+ astrocytes of the neonatal/perinatal spinal cord. Importantly, despite their known Olig2 developmental requirement, WM-AS were not more likely to express Olig2 than GM-AS were. This expression pattern indicates that Olig2 may play an uncharacterized developmental role in immature GM astrocyte subpopulations of the SC.

Effects of Olig2-CKO in GFAP+ AS of the spinal cord.

Heterozygous conditional deletion of Olig2 caused aberrant expression of oligodendrocyte-lineage markers in AS-lineage cells. Homozygous conditional knockout of Olig2 produced mixed results. The Olig2-CKO spinal cords each showed one of two patterns: either a) no noticeable effect on astrocyte number compared to control, or b) an almost-complete lack of labeled astrocytes. The fact that a subset of the Olig2-CKO spinal cords produced results in accordance with the hypothesized outcome is interesting; however, additional data is required before any conclusion can be drawn from this component of the experiment

References

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[2] Vue et al. (2014). Development. 141(19): 3721-3731.
[3] Kettenmann and Ransom. (2013). *Neuroglia*, pp. 114.
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