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Prox1 Is Required for Oligodendrocyte Cell Identity in Adult Neural Stem Cells of the Subventricular Zone

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ABSTRACT

Adult neural stem cells with the ability to generate neurons and glia cells are active throughout life in both the dentate gyrus (DG) and the subventricular zone (SVZ). Differentiation of adult neural stem cells is induced by cell fate determinants like the transcription factor Prox1. Evidence has been provided for a function of Prox1 as an inducer of neuronal differentiation within the DG. We now show that within the SVZ Prox1 induces differentiation into oligodendrocytes. Moreover, we find that loss of Prox1 expression in vivo reduces cell migration into the corpus callosum, where the few Prox1 deficient SVZ-derived remaining cells fail to differentiate into oligodendrocytes. Thus, our work uncovers a novel function of Prox1 as a fate determinant for oligodendrocytes in the adult mammalian brain. These data indicate that the neurogenic and oligodendrogliogenic lineages in the two adult neurogenic niches exhibit a distinct requirement for Prox1, being important for neurogenesis in the DG but being indispensable for oligodendrogliogenesis in the SVZ. STEM CELLS *2016;34:2115–2129*

SIGNIFICANCE STATEMENT

In the submitted study, we address the function of the homeobox transcription factor Prox1 for the specification of oligodendrocyte cell fate in adult neural stem cells. A function of Prox1 for neurogenesis is well described in Drosophila. Additionally, recently its implication in neuronal differentiation in neural stem cells has been shown. Therefore, the function seemed to be totally conserved from Drosophila to mammals. However, we here show that the function of Prox1 depends on the regional identity of the investigated neural stem cells. In neural stem cells of the hippocampus, Prox1 induces neuronal differentiation. However, in neural stem cell from the subventricular zone Prox1 induces differentiation in oligodendrocytes.

INTRODUCTION

Adult neurogenesis has been shown to occur in at least two specific areas of the brain, the subgranular zone of the hippocampal dentate gyrus (DG) and the subventricular zone (SVZ) of the lateral ventricle (LV) in mammals, primates, and humans [1–4]. Newly generated cells of the DG mature into granule neurons of the granular cell layer while cells generated in the SVZ migrate along the rostral migratory stream (RMS) into the olfactory bulb (OB) [5, 6]. However, it was also shown that a subset of cells generated in the adult SVZ migrate into the corpus callosum (cc) to give rise to oligodendrocytes [7]. During forebrain development, oligodendrogenesis starts at embryonic day 12.5 in two waves within the medial and lateral ganglionic eminence. There, sonic hedgehog signaling induces activation of the homeodomain transcription factor Nkx6 and the basic helix-loop-helix transcription factor Olig2 [8, 9]. While sonic hedgehog signaling seems to be activated by Sulfatase 1 secretion [10]. Finally, a third wave of oligodendrocyte generation emerges in early postnatal stages from cortex-derived precursor cells [11, 12]. While the original Nkx6-positive oligodendrocytes are gradually eliminated from the brain and completely disappear after birth, the cortex-derived cells migrate from their site of origin to colonize gray and white matter of the brain [11, 13]. Recently, the transcription factors Ascl1, Sox10, and Nkx2.2 have been

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shown to regulate oligodendrocyte specification from neural stem cells [14]. Among these factors, Ascl1 is particularly interesting because it controls the number and distribution of astrocytes and oligodendrocytes in the gray matter and white matter of the spinal cord [15]. Within the adult brain, newly generated oligodendrocytes arise from two distinct sources,

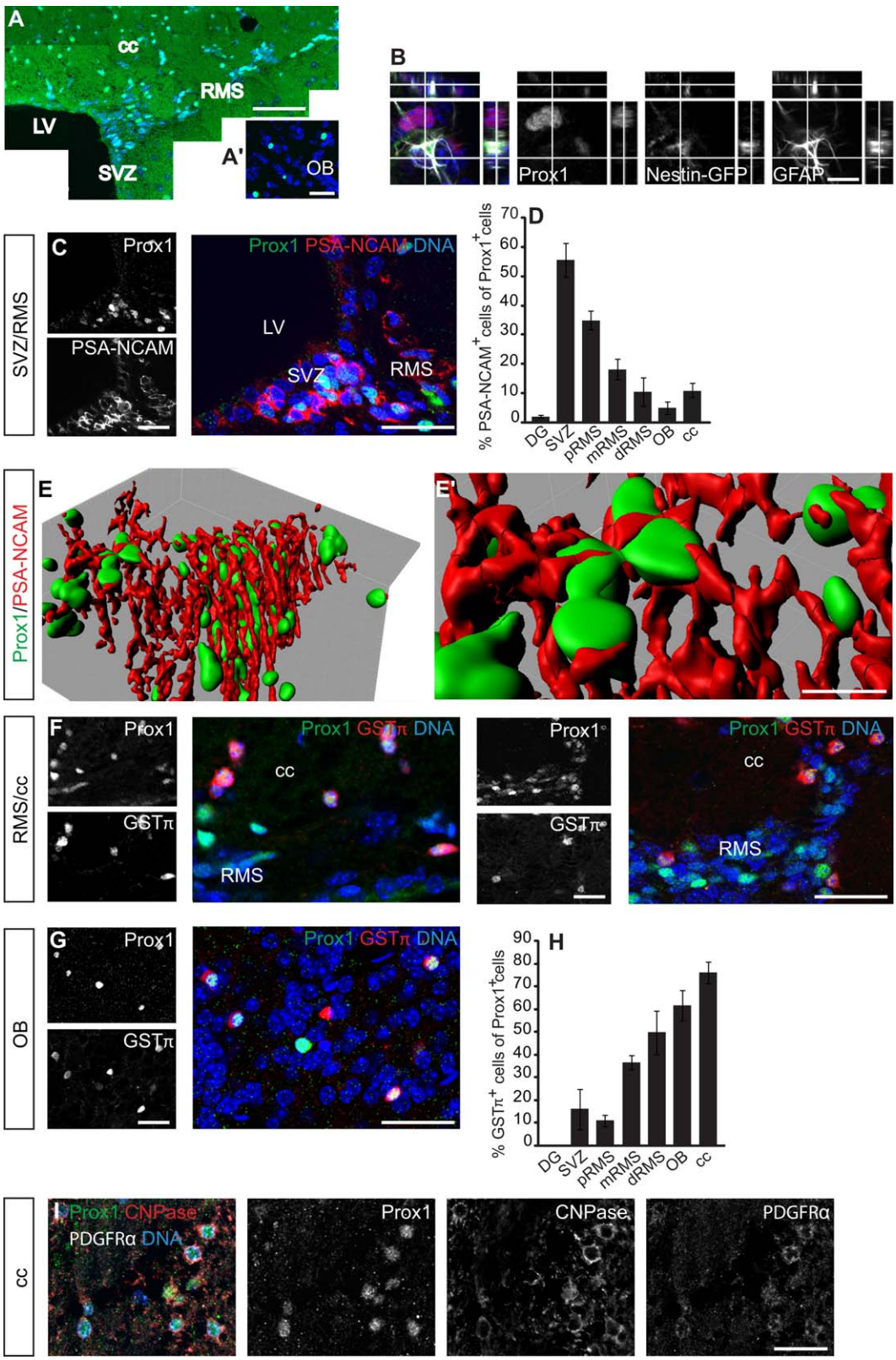

Figure 1.

the local parenchymal oligodendrocyte precursor cells (OPCs) and the SVZ neural stem cells. Parenchymal OPCs were shown to reflect a morphologically heterogeneous population of NG2-positive cells. These cells continue to proliferate into adulthood both under physiological conditions [16] as well as following demyelinating lesions [17, 18]. Within the SVZ, it has been suggested that a subset of Olig2-expressing transient amplifying cells (Type C cells) may give rise to highly migratory OPCs that leave the SVZ and populate the cc, striatum, and fimbria fornix, where they differentiate into NG2-positive glia and myelinating oligodendrocytes [19]. These results suggest that OPCs are not only continuously generated within the SVZ but are also increased following demyelinating lesions where they are involved in repair processes [7, 20]. Interestingly, although the parenchymal OPCs within the cc also generate new oligodendrocytes, these cells seem to differentiate only close to their location of origin while OPCs from the SVZ have the ability to disperse along the cc [7] making them to a highly valuable therapeutic target.

Prox1 functions as a homeobox transcription regulator. It was shown to be transiently expressed during embryonic development within heart, liver, pancreas, and the CNS, suggesting a role in cell differentiation [21–23]. With regard to the developing mammalian CNS, Prox1 expression was found in locations where neurogenesis and gliogenesis occurs and where it appears to control cell cycle progression [24]. Recently, it has been shown that in mice Prox1 regulates the postnatal specification of caudal ganglionic eminence-derived GABAergic interneurons [25].

However, the function of Prox1 in the adult mammalian brain is largely unknown. Within the adult brain, remaining expression of Prox1 was only found in hippocampal granule neurons in the DG as well as in cells of the internal granular layer and white matter of the cerebellum [24]. Recently, first evidence was provided for a function of Prox1 within the adult hippocampus where it seems to be required for proper neuronal differentiation of hippocampal neural stem cells [26]. Now, we report that Prox1 expression also persists within the SVZ where neural stem cells reside and continue to give rise to new cells of the glial and neuronal lineage. Moreover, our results demonstrate that Prox1 is necessary to induce oligodendrocyte differentiation in SVZ derived progenitor cells. Furthermore, we provide evidence that Prox1 interacts with and promotes the expression of *notch1* and *olig2* thereby regulating oligodendrocyte differentiation.

Our results imply fundamental differences in the differentiation potential of adult neural stem cells from the DG in comparison to those from the SVZ. Additionally, they highlight Prox1 as a novel therapeutic target in promoting oligodendrocyte differentiation in demyelinating diseases.

Materials and Methods

For information on material and methods, see Supporting Information.

Results

Prox1 Is Expressed in Type A Progenitor Cells of the SVZ and in Oligodendrocytes of the OB, cc, and Cortex

To analyze whether expression of the transcription regulator Prox1 was not only associated with the hippocampal DG but also with a second well described adult neurogenic niche, the SVZ, we performed immunohistochemical labeling of sagittal brain sections. Unexpectedly, we observed a robust expression of Prox1 in the SVZ and along the RMS (Fig. 1A) while the amount of Prox1-positive cells was extremely low in the OB (Fig. 1A′, Supporting Information Fig. S1A). In contrast, 70% of all cells were positive for Prox1 in the cc (Fig. 1A, Supporting Information Fig. S1A). As previously described [27], Prox1 was detected in around 90% of the cells within the DG (Supporting Information Fig. S1A). To further analyze which cell types expressed Prox1, we performed co-immunolabeling experiments. Analysis of brain sections generated from mice expressing GFP under the control of the Nestin promoter revealed that Nestin/GFAP double-positive neural stem cells within the SVZ did not express Prox1, suggesting that Prox1 expression is not associated with type B neural stem cells in the SVZ (Fig. 1B). Additionally, only very few Prox1-positive cells within the SVZ and the proximal RMS (pRMS) expressed the type C cell marker Mash1 (Supporting Information Fig. S1B, S1D). However, around 55% of Prox1-positive cells within the SVZ were positive for PSA-NCAM suggesting that Prox1 expression is mainly associated with type A progenitor cells (Fig. 1C, 1D, 1E, 1E′). Along the RMS we observed a decrease in the number of Prox1/PSA-NCAM coexpressing cells to around 5% within the OB and 10% within the cc (Fig. 1D). Strikingly, while Prox1-positive cells within the DG expressed the neuronal marker protein, NeuN, Prox1-positive cells in the OB did not differentiate into the neuronal lineage (Supporting Information Fig. S1C, S1E). To further elucidate the identity of Prox1-positive cells within different brain regions immunolabeling with the oligodendrogial marker, GSTπ, CNPase, and

Figure 1. Prox1 expression in the adult SVZ is associated with PSA-NCAM-positive progenitor cells and with oligodendrocyte marker within the corpus callosum and olfactory bulb. **(A, A′)**: Sagittal brain sections of wild-type adult mice were stained for Prox1 (green) and Prox1-positive cells were found within the SVZ, along the RMS and in the OB. **(B)**: Nestin-GFP mice were analyzed for Prox1 expression. Sagittal mouse brain sections were stained by immunohistochemistry for Prox1 (red), GFAP (white), and GFP (green). Nestin/GFAP-positive cells within the SVZ, representing the pool of neural stem cells did not express Prox1. **(C-I)**: Sagittal brain sections of wild-type adult mice were stained for Prox1 (green) and PSA-NCAM, GSTπ, or CNPase (red) and PDGFRα (white). (C, D): Prox1-positive cells expressing PSA-NCAM were mainly found within the SVZ and proximal pRMS (E, E′) 3D reconstruction of the image shown in (C). (E′): Higher magnification from (E). Within the cc (F) and the OB (G) Prox1-positive cells express GSTπ. (D, H): The number of PSA-NCAM or GSTπ-expressing cells among the Prox1-positive cells was quantified in different regions of the brain sections. Data are represented as mean ± SEM. Scale bars: (A) 70 μm, (A′) 50 μm, (B) 5 μm, (C) 50 μm, (E′) 10 μm, (F, G) black and white images 25 μm and color images 40 μm, (I) 30 μm. Abbreviations: cc, corpus callosum; DG, dentate gyrus; dRMS, distal RMS; mRMS, middle RMS; GFP, green fluorescent Protein; GFAP, glial fibrillary acidic protein; LV, lateral ventricle; OB, olfactory bulb; pRMS, proximal RMS; PSA-NCAM, poly-sialated neural cell adhesion molecule; RMS, rostral migratory stream; SVZ, subventricular zone.

PDGFRα or Nogo-A was performed. We observed a high number of Prox1/GSTπ-positive cells within both regions, the cc and OB (Fig. 1F, 1G, 1H). Furthermore, also coexpression of Prox1 with CNPase and PDGFRα was found in corpus callosal cells (Fig. 1I). In addition, both in the cortex and in the cc a subset of Nogo-A-positive cells expressed Prox1 (Supporting Information Fig. S1F, S1G), all in all confirming that Prox1-positive cells are indeed oligodendrocytes.

Prox1 Is Predominantly Expressed within the Dorsal Regions of the LV Wall along the Anterior-Posterior Axis of the Brain

Previously, Merkle et al. have shown that neural stem cells from the SVZ are organized in a complex mosaic pattern [28]. They showed that localization within this neurogenic region influences which types of neurons they can generate. Therefore, it was concluded that the cells must have integrated positional information during development. To analyze whether Prox1 expression was associated with specific areas of the SVZ, six different regions along the anterior-posterior axis (defined as anterior-posterior coordinates from Bregma) were investigated by immunohistochemistry on coronal brain sections (Supporting Information Fig. S2). Within each region, the SVZ was subdivided into a medial (septal) (M), ventral (V), lateral (L), and dorsal (subcallosal) (D) area and the number of Prox1 expressing cells was quantified and given as a percentage of the total cell number. Additionally, the distal RMS (dRMS, in region 1) and the dorso-ventral extent of the SVZ (in region 6) were included ($N = 4$ mice, $n = 3$-8 fields per area). Interestingly, the dorsal area of the SVZ showed increased Prox1 expression compared to all other areas (Supporting Information Fig. S2B). Statistical analyses revealed that Prox1 expressing cells were significantly increased in the dorsal areas of region 2 to 5 over the ventral areas as well as over the medial area in region 2 and the lateral area in region 4 (region 2: area D [47.1 ± 10.8%] vs. area V [9.8 ± 1.8%] $p = .02$, area D vs. area M [7.7 ± 2.4] $p = .007$; region 3: area D [19.8 ± 1.7%] vs. area V [5.7 ± 1.4%] $p = .001$; region 4: area D [39.8 ± 3.6%] vs. area L [20.3 ± 4.2%] $p = .02$, area D vs. area V [3.5 ± 1.1%] $p = .001$; region 5: area D [33.6 ± 6.8%] vs. area V [6.8 ± 1.5%] $p = .03$). Additionally, in regions 2 and 5, the lateral area exhibited significantly more Prox1-positive cells when compared to the ventral area (region 4: area L vs. V $p = .008$; region 5: area L vs. V $p = .02$). In the dRMS (region 1) as well as the dorso-ventral extent of the SVZ (region 6), only few Prox1 expressing cells were found with 4.4 ± 0.8% and 4.2 ± 1.4% cells, respectively (Supporting Information Fig. S2C). Overall, these data show that Prox1 is predominantly expressed within the dorsal area of the LV wall, which is the one most proximate to the cc. It is therefore tempting to speculate that this expression pattern reflects the function of Prox1 in oligodendrogenesis for the cc.

Prox1 Expressing Cells Within the cc Are Adult Generated Oligodendrocytes while Prox1-positive cells in the OB Are Not Generated via Adult Neurogenesis

To further validate whether Prox1-positive cells within the different brain regions were adult generated, mice were injected with 25 mg/kg 5-bromo-2′-deoxyuridine (BrdU) twice daily for 3 consecutive days and sacrificed 2, 5, 8, 15, or 21 days following the last injection. Cells that incorporated BrdU were analyzed for Prox1 expression and NeuN or GSTπ (Fig. 2). As previously described, we found that as early as 2 days following BrdU administration already 74.4 ± 3.5% BrdU-positive cells of the DG expressed Prox1, consistent with the notion that Prox1 is important for neural progenitor cells of this neurogenic niche [26]. Three days later, at 5 days following BrdU injection 100% of BrdU-positive cells were also Prox1-positive ($BrdU^+/Prox1^+$ cells 2 days vs. 5 days following BrdU $p = .008$) (Fig. 2A, 2B). Inversely, within the SVZ BrdU/Prox1-positive cells decreased from 94.7 ± 3.5% at day 2 to 41.7 ± 18.6% at day 21, possibly due to the fact that compared to the DG newly generated cells from the SVZ migrate into RMS and cc. Interestingly, also in the cc already at day 2, 74.5 ± 4.7% of BrdU incorporated cells expressed Prox1 which further increased over time to 90.7 ± 3.4% at day 21 following BrdU administration confirming that Prox1-expressing cells in the cc are indeed adult generated. Surprisingly, at no time point BrdU/Prox1 double-positive cells were detectable within the OB (Fig. 2A, 2B). We, therefore, conclude that Prox1 has different functions in the specification of oligodendrocytes and neurons from the SVZ. While it is only expressed transiently in the neuronal lineage, expression of Prox1 stays on in the oligodendrocyte lineage (see Supporting Information Table S1).

Additionally, the number of BrdU/Prox1-double-positive cells that developed into neurons was analyzed by NeuN coexpression (Fig. 2C, 2E). As expected within the DG as early as 2 days following BrdU administration no BrdU/Prox1-positive cells expressed NeuN suggesting that they were still in an undifferentiated progenitor state. However, already 5 days following BrdU injection 72.1 ± 17.3% of the BrdU/Prox1 double-positive cells coexpressed NeuN ($NeuN^+$ cells of $BrdU^+/Prox1^+$ cells 2 days vs. 5 days following BrdU $p = .01$). Since no BrdU/Prox1-double-positive cells were found in the OB (Fig. 2A, 2B) it was not surprising that we were unable to detect newly generated neurons expressing Prox1 over all time points analyzed (Fig. 2C, 2E), suggesting that Prox1 expression in newly generated cells from the SVZ is only transiently associated with adult neurogenesis for the OB. Additionally, despite the fact that in the cc up to 90% of the BrdU-positive cells expressed Prox1, not a single BrdU/Prox1-double-positive cell became a NeuN-positive neuron (Fig. 2C, 2E). Finally, we used GSTπ immunostaining to address the question whether oligodendrocytes were generated from BrdU/Prox1-double-positive cells (Fig. 2D, 2F). Within the two neurogenic niches, DG and SVZ, as well as the OB no BrdU-positive cells were found coexpressing Prox1 and GSTπ (Fig. 2D, 2F). However, when we analyzed BrdU/Prox1-double-positive cells within the cc we found that while up to 8 days following BrdU administration no cells had developed into oligodendrocytes, from day 15 on the cells started to express GSTπ indicating their oligodendrocytic identity (Fig. 2D, 2F). At 15 days following BrdU injection, 26.1 ± 8.1% of the BrdU/Prox1-double-positive cells were also positive for GSTπ. This fraction increased to 35.6 ± 6.7% at day 21 ($GST\pi^+$ cells of $BrdU^+/Prox1^+$ cells 8 days vs. 21 days following BrdU $p = .002$). Additionally, 21 days following BrdU injection the BrdU/Prox1-double-positive cells were also positive for APC, which further confirmed their oligodendrocytic nature (Supporting Information Fig. S3A, S3B). Taken together, we therefore conclude that while newly generated cells which continuously express Prox1 generate neurons for the DG, they generate oligodendrocytes for the cc.

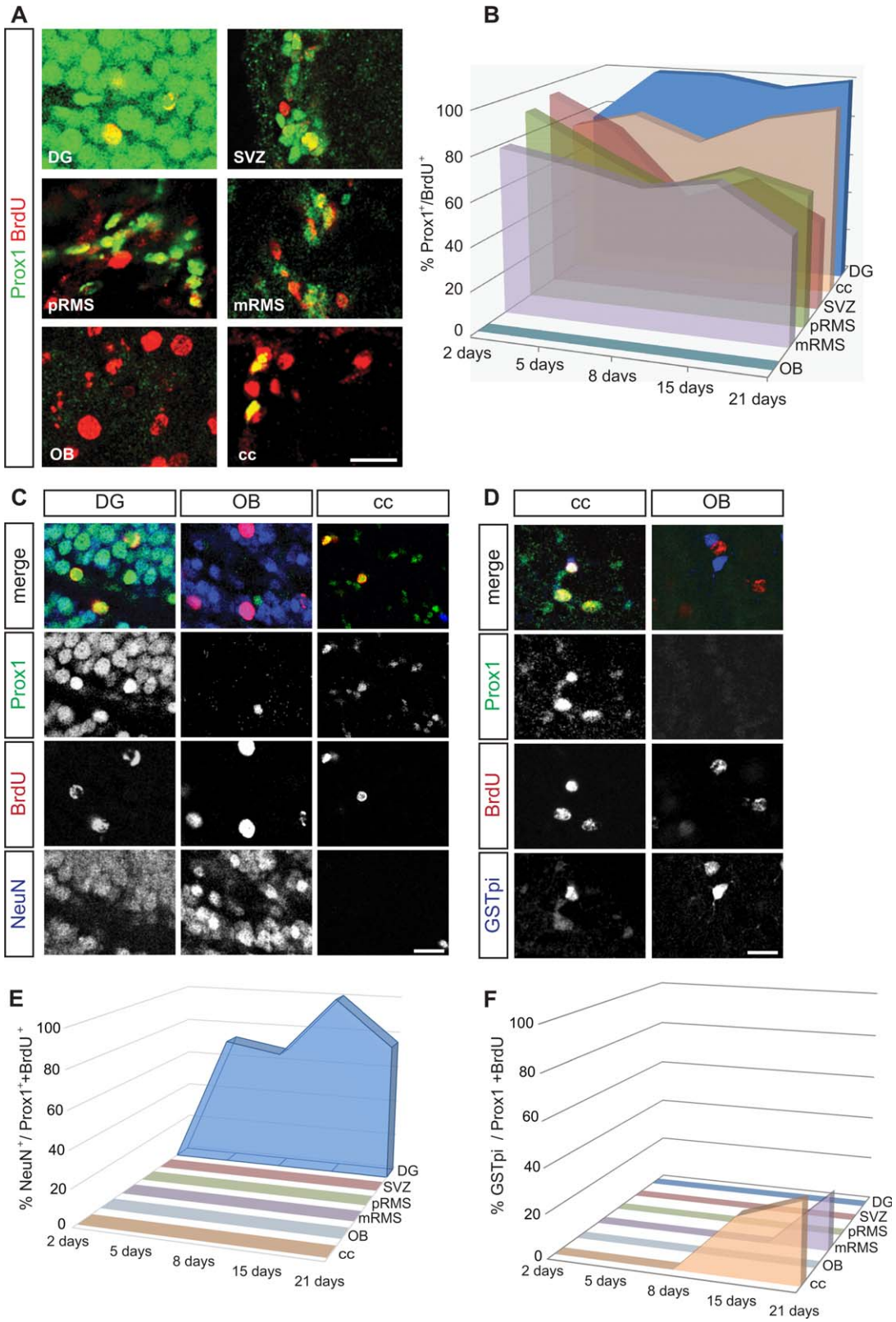

Figure 2. Adult generated Prox1 expressing cells become oligodendrocytes in the corpus callosum. **(A)**: Representative images of immunohistochemistry with Prox1 (green) and BrdU (red) from sagittal brain sections within indicated brain regions of BrdU treated mice. **(B)**: Quantification of Prox1/BrdU-double-positive cells within indicated regions at different time points after BrdU treatment. **(C, D)**: Triple staining using BrdU (red), Prox1 (green) and NeuN or GSTπ (blue) of sagittal brain sections at day 21 following BrdU injection is shown. **(E, F)**: Quantification of NeuN or GSTπ expressing Prox1-positive newborn cells within indicated regions at different time points after BrdU injection. Data are represented as mean. Scale bars: (A, C, D) 20μm. $N = 3$ mice per time point. Abbreviations: cc, corpus callosum; DG, dentate gyrus; mRMS, middle RMS; OB, olfactory bulb; pRMS, proximal RMS; SVZ, subventricular zone.

Adult Generated Cells within the cc Originate from the SVZ, Develop into Oligodendrocytes and Express Prox1

To investigate whether adult generated cells within the cc originate from progenitor cells of the SVZ or from locally proliferating cells, we performed stereotaxic injections with a retrovirus carrying a GFP expression cassette. To only label proliferating cells within the SVZ, the virus was specifically injected into this region and animals were sacrificed 8 days

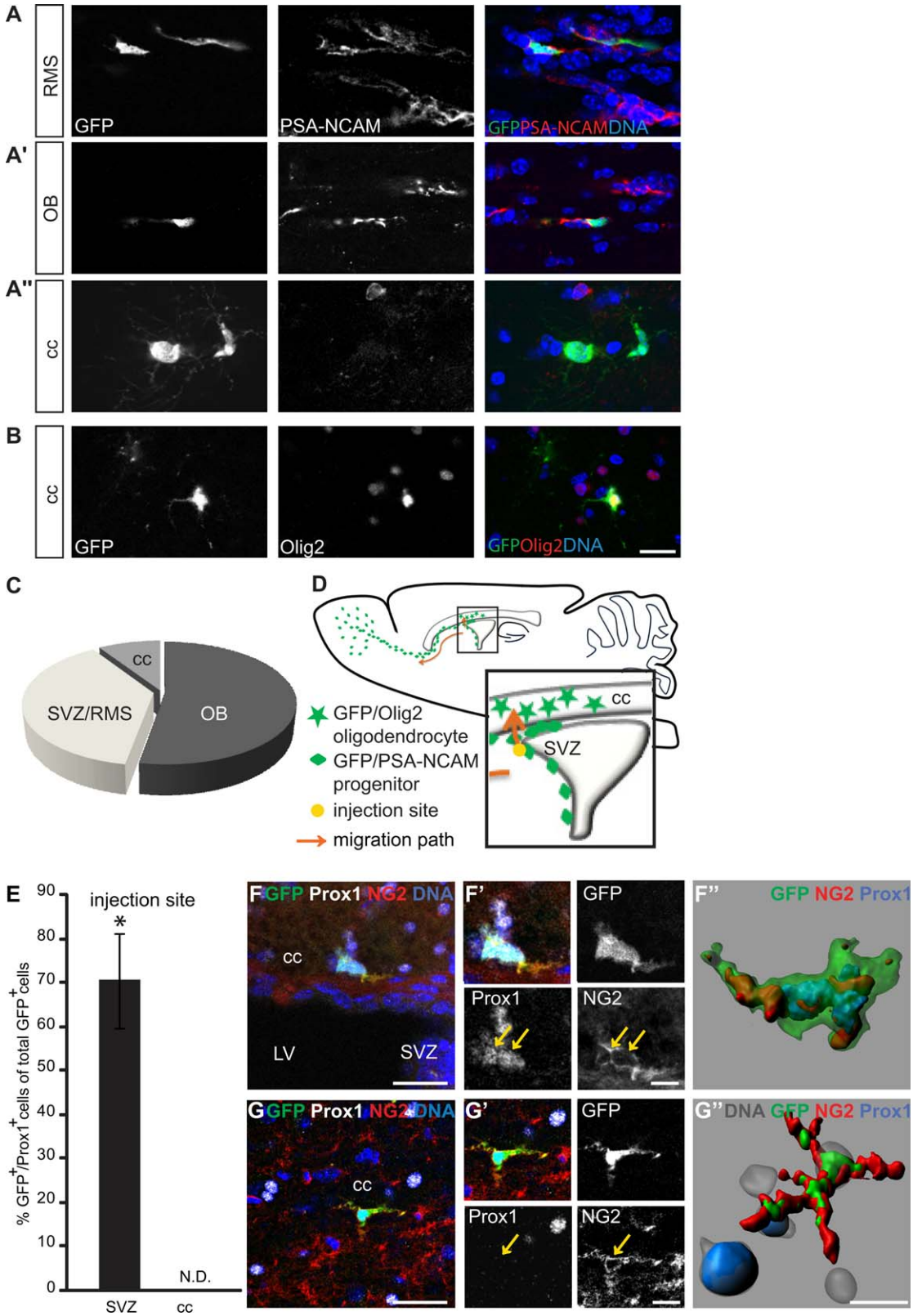

Figure 3.

following surgery. Analysis of the distribution of GFP-expressing cells revealed that while around 40% and 50% of the cells where located within the SVZ/RMS region or the OB, respectively, another 10% of the cells had migrated into the cc suggesting that adult generated cells within the cc indeed originate from the SVZ (Fig. 3C, 3D). Co-immunolabeling of sagittal brain sections revealed that GFP-virus transduced cells along the SVZ/RMS and in the OB expressed PSA-NCAM, in contrast to the cells within the cc (Fig. 3A-A″, 3D). Cells within the cc were positive for Olig2 indicating that they had differentiated into oligodendrocytes (Fig. 3B, 3D).

It has been shown previously that not only SVZ-derived cells can give rise to oligodendrocytes, but also that parenchymal OPCs proliferate locally in the cc [7, 18]. Therefore, to investigate whether we could distinguish between these two populations of cells, a retrovirus carrying a GFP expression cassette was stereotaxically injected either into the SVZ or into the cc and GFP-expressing cells in the cc were analyzed for Prox1 expression (Supporting Information Fig. S4). Indeed, while SVZ-derived cells in the cc showed Prox1 immunostaining (Supporting Information Fig. S4, lower panel) Prox1 expression was absent from locally proliferating OPCs in the cc (Supporting Information Fig. S4, upper panel). Statistical analysis revealed that $70.6 \pm 10.8\%$ of GFP-positive cells from the SVZ expressed Prox1 in the cc while for cells transduced in the cc (representing local OPCs) we were unable to find a single cell expressing Prox1 (Fig. 3E, $N = 7$ mice (cc injection, total cell number counted in the cc = 59 cells) and three mice (SVZ injection, total cell number counted in the cc = 109 cells), $n = 3$-6 sections per mouse). Finally, costaining with Prox1 and NG2 confirmed that GFP-expressing cells from the SVZ not only expressed Prox1 but also developed into oligodendrocytes as verified by NG2 expression (Fig. 3F). Also, locally transduced cells in the cc expressed NG2 confirming that these cells were indeed parenchymal OPCs (Fig. 3G). Overall, these data suggest that Prox1 expression is specific to newly generated oligodendrocytes for the cc that are derived from neural stem cells of the SVZ while Prox1 is absent from local OPCs. However, formally we cannot fully exclude that OPC subtypes that have a very long cell cycle time are missed within the here described approach. Although taking this potential limitation into account, to our knowledge, therewith Prox1 represents the first marker allowing to distinguish between SVZ-derived oligodendrocytes and local OPC-derived oligodendrocytes.

Prox1 Is Expressed in the Oligodendrogliogenic Lineage in Primary Cultures of the SVZ

Previously, it was shown that clonal founder cells in primary cultures of the SVZ produce either neuronal or oligodendroglial progeny suggesting that neural stem cells in these cultures constitute strictly separated lineages [29]. To analyze the involvement of Prox1 in oligodendrogenesis in vitro, primary cultures were prepared from the dorsal and lateral walls of the LV of adult mice and single cell tracking was performed by continuous live imaging as described previously by Ortega et al. (2013), followed by immunocytochemistry for oligodendroglial or neuronal marker expression (PDGFR α or β III Tubulin, respectively) as well as for Prox1 (Fig. 4, Supporting Information Videos 1 and 2). In accordance with our data in vivo, cells within oligodendrogliogenic clones were positive for Prox1 expression (Fig. 4A-4C), while within neurogenic clones, Prox1 expression occurred fairly restricted and often could not be detected (Fig. 4D-4F), consistent with a more prominent role of Prox1 in oligodendrogenesis. In fact, within neurogenic clones, Prox1 expression appeared to be transient. While early stage neuroblasts located within the core of clonal cell clusters showed intense Prox1 expression (Supporting Information Fig. S5B, inset 1), later stage neuroblasts that had migrated out from their cluster following commencement of migratory activity Prox1 expression was weak or absent (Supporting Information Fig. S5B, inset 2). The dynamic Prox1 expression during neurogenic lineage progression in these clonal cultures is similar to the transient Prox1 expression observed in early neuroblasts in the SVZ followed by a downregulation in neurons of the OB in vivo. In contrast, Prox1 expression remained high throughout oligodendroglial lineage progression both in vitro as well as in vivo.

Prox1 Is Expressed in Neural Stem Cell Cultures and Induces Oligodendrocyte Differentiation In Vitro

To further elucidate whether Prox1 is involved in the differentiation into oligodendrocytes of conventional neural stem cell cultures [30], (NSCs) Neural Stem Cell were kept under multilineage differentiation conditions for 3 or 7 days (allowing the cells to become astrocytes, neurons, or oligodendrocytes) and the cells were analyzed for Prox1 and O4, GFAP or Tuj1 expression (Supporting Information Fig. S6A-S6C). Interestingly, while Prox1-positive cells expressed either Tuj1 or O4 after 3 days of differentiation (3 days differentiation

Figure 3. Adult generated cells of the SVZ migrate into the corpus callosum and Prox1 expression is specific to oligodendrocytes generated from these cells. **(A-D)**: A GFP-carrying retrovirus was injected stereotactically into the SVZ of adult mice and animals were sacrificed 8 days following surgery ($N = 11$ mice, $n > 1400$ cells). (A, B): Sagittal brain sections were stained by immunohistochemistry for GFP (green) and PSA-NCAM or Olig2 (red) and different brain regions were analyzed. (A, A′): GFP-positive cells were found along the RMS and within the OB coexpressing PSA-NCAM. GFP-positive cells within the cc did not express PSA-NCAM (A″) but were positive for the oligodendrocyte marker Olig2 (B). (C): The number of GFP-positive cells was quantified in different regions of the brain. Ten percent of GFP-expressing cells originating from the SVZ were found within the cc (light gray slice), 40% were located along the SVZ/RMS (white slice) and 50% were found in the OB (dark gray slice). (D): Cartoon representing the migration pattern of transduced progenitors from the SVZ into the RMS/OB (green diamond) and cc (green stars). **(E-G″)**: Retroviruses carrying a GFP expression cassette were injected into the SVZ or cc and GFP-positive cells were analyzed in the cc. (E) Quantification of GFP-positive cells expressing Prox1 in the cc. Among cells generated from the SVZ, $70.6 \pm 10.8\%$ express Prox1 while in local OPCs Prox1 was not detected. (F, F′, G, G′): Immunohistochemistry of GFP (green), Prox1 (white), and NG2 (red) in the cc following viral transduction of SVZ cells (F, F′) or local OPCs (G, G′). (F″, G″): 3D reconstruction of cells depicted in (F) and (G), respectively (DNA, gray; GFP, green; NG2, red; Prox1, blue). For better illustration, images were rotated. SVZ-derived cells express Prox1 and NG2 while local OPCs express only NG2. Data are represented as mean ± SEM. *$p = .02$. Scale bars: (A, B) 25 μm, (F, G) 30 μm, (F′, F″, G′, G″) 10 μm. Abbreviations: cc, corpus callosum; GFP, green fluorescent protein; N.D., not detected; OB, olfactory bulb; OPC, oligodendrocyte precursor cell; PSA-NCAM, poly-sialated neural cell adhesion molecule; RMS, rostral migratory stream; SVZ, subventricular zone.

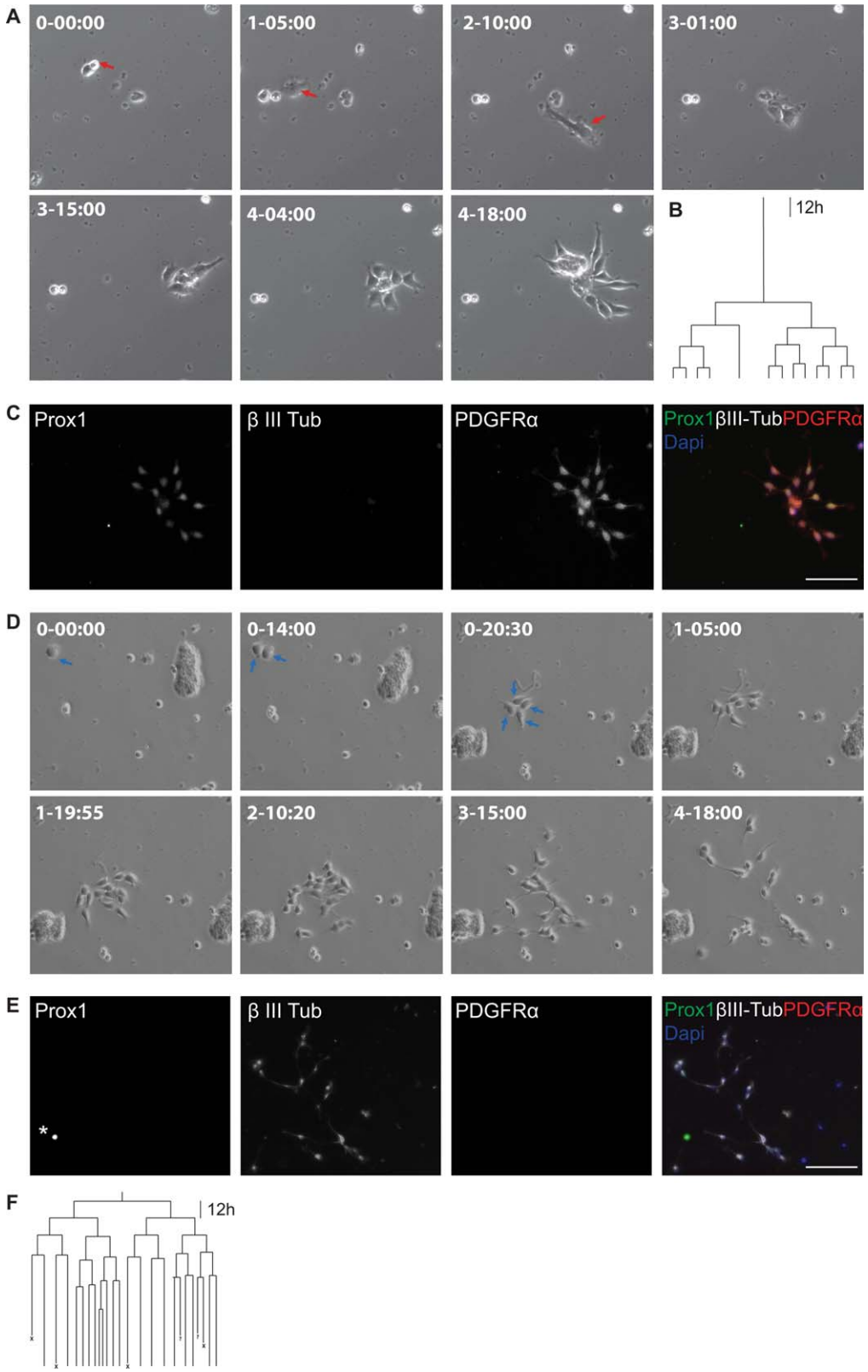

Figure 4. Prox1 is expressed within the oligodendrogliogenic lineage in primary cultures of the subventricular zone. Neuro (blue arrows in **D-F**) and oligodendrogliogenic (red arrows in **A-C**) lineage trees tracked by live imaging in cultures of the dorsal and lateral walls of the lateral ventricle of adult mice. Phase contrast images obtained by time-lapse video microscopy at different time points [day-hour:minute; (A) oligodendrogliogenic lineage; (D) neuronal lineage); images in (C) and (E) represent postimaging immunocytochemistry for βIII-tubulin (white), PDGFRα (red), Prox1 (green), and Dapi (blue) of lineage trees tracked in (A) and (D), respectively. *Staining artifact in the Prox1 channel as verified by absence of nuclear Dapi staining. (B, F): Lineage trees derived from the live imaging experiment depict oligodendrogliogenic and neurogenic clones, respectively.

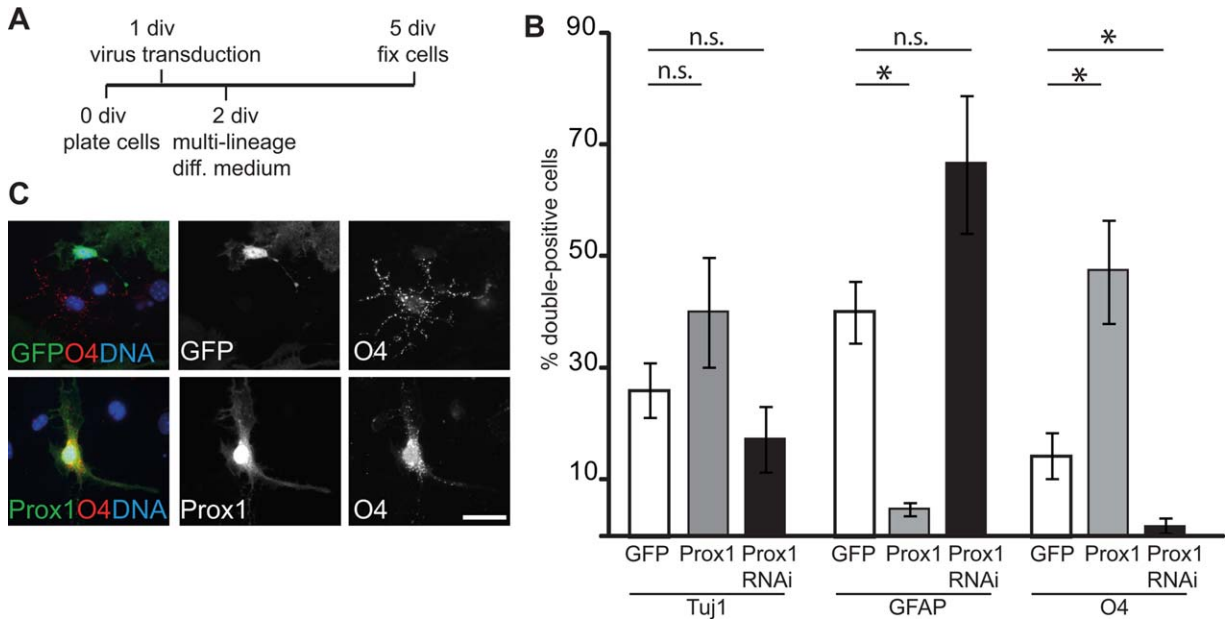

Figure 5. Prox1 is necessary for oligodendrocyte differentiation of neural stem cells in vitro. Neural stem cells were transduced using GFP, Prox1-GFP, or Prox1-RNAi-GFP retroviruses and cells were kept under multilineage differentiation conditions. Following 3 days, the cells were fixed and stained for Tuj1, GFAP, or O4. **(A)**: Experimental outline. **(C)**: Immunocytochemistry for GFP (green) and O4 (red). The majority of cells transduced with the GFP virus did not differentiate into O4-positive oligodendrocytes (upper panel). Prox1 over-expression induced oligodendrocyte differentiation (lower panel). **(B)**: Quantification of cell differentiation into neurons (Tuj1), astrocytes (GFAP), or oligodendrocytes (O4) following viral transduction. Data are represented as mean ± SEM. Values represent the mean of 7 individual experiments. *$p = .02$, Scale bar: (C) 25 μm. Abbreviations: GFP, green fluorescent protein; GFAP, glial fibrillary acidic protein; n.s., not significant.

68.2 ± 6.1% Tuj1-positive, 66.9 ± 6.6% O4-positive; 7 days differentiation 53.1 ± 9.5% Tuj1-positive, 79.7 ± 7.1% O4-positive) Prox1/GFAP-double-positive cells were found only in 1.42 ± 0.8% or 7.81 ± 4.6% 3 or 7 days following differentiation, respectively (Supporting Information Fig. S6D, S6E). Furthermore, in O4-positive cells Prox1 expression was predominantly found in the nucleus (Supporting Information Fig. S6F, O4 pos. cells, nuclear vs. cytosolic $p = .004$) whereas within the population of cells that were negative for O4, Prox1 was mainly localized in the cytoplasm (Supporting Information Fig. S6F, O4 neg. cells, nuclear vs. cytosolic $p = .004$). Interestingly, already previously it has been shown for cyclin-dependent kinase inhibitors that besides their expression, also their subcellular localization regulates cell fate.

Moreover, Prox1 was also expressed in primary cultures of oligodendrocytes derived from newborn mice (Supporting Information Fig. S7). While immature oligodendrocytes (verified by A2B5 expression) 1 day in vitro (div) showed Prox1 staining both in cytoplasm and nucleus (Supporting Information Fig. S7A), following maturation for 3 and 5 div (verified by O1 and MBP expression, respectively) Prox1 became restricted to the nucleus similarly to what was observed in neural stem cells (Supporting Information Fig. S7B, S7C).

Under multilineage differentiation conditions, neural stem cells develop neuronal as well as glial phenotypes. Therefore, we analyzed whether loss or gain of Prox1 function would alter the cell's commitment to one or the other lineage. Neural stem cell cultures were infected with retroviruses carrying expression cassettes for GFP, Prox1 and GFP, or Prox1-RNAi and GFP. Transduced cells were kept under multilineage differentiation conditions designed to augment oligodendrocyte differentiation for 4 days (Fig. 5A) [34]. Under control conditions (expression of GFP) around 26% of the cells differentiated into neurons as shown by Tuj1 staining, while 40% of the cells became GFAP-positive astrocytes and around 14% became O4-positive oligodendrocytes (Fig. 5B). However, following the overexpression of Prox1 significantly more neural stem cells differentiated into oligodendrocytes (control vs. Prox1, O4-positive cells, $p = .002$) while there was a significant reduction in the number of astrocytes (control vs. Prox1, GFAP-positive cells, $p = .002$) suggesting that Prox1 expression induces oligodendrocyte identity in neural stem cells at the expense of astrocyte differentiation (Fig. 5B, 5C). Interestingly, the reverse effect was observed following Prox1 knockdown. The loss of Prox1 expression resulted in a slight, however, not significant increase in the number of astrocytes while the number of O4-positive oligodendrocytes was significantly reduced (control vs. Prox1-RNAi, O4-positive cells, $p = .02$) and almost absent from the culture (Fig. 5B). Overexpression or knockdown of Prox1 resulted in only nonsignificant changes in the number of Tuj1-positive neurons suggesting that Prox1 does not play a determining role in neuronal cell fate commitment within this neural stem cell culture (Fig. 5B).

Loss of Prox1 Function in SVZ Progenitors Results in Decreased Levels of Oligodendrocytes for the cc

So far, our data suggest that Prox1 plays an important role in adult generation of oligodendrocytes from SVZ-derived neural stem cells, thus, we wondered whether knockdown of Prox1 in vivo would alter the differentiation characteristics of these cells. Therefore, retroviruses carrying either an expression cassette for GFP or for Prox1-RNAi and GFP were injected into the SVZ to transduce proliferating cells. For analysis, sagittal brain sections were stained 8 days following surgery (Fig. 6).

Interestingly, we found a strong reduction in the relative amount of transduced cells within the cc when Prox1 expression was knocked down (control vs. Prox1-RNAi, $p = .004$) suggesting that Prox1 is important for cell migration into the cc (Fig. 6A, 6E, 6F). However, no differences were observed in the relative amount of transduced cells within the RMS or OB

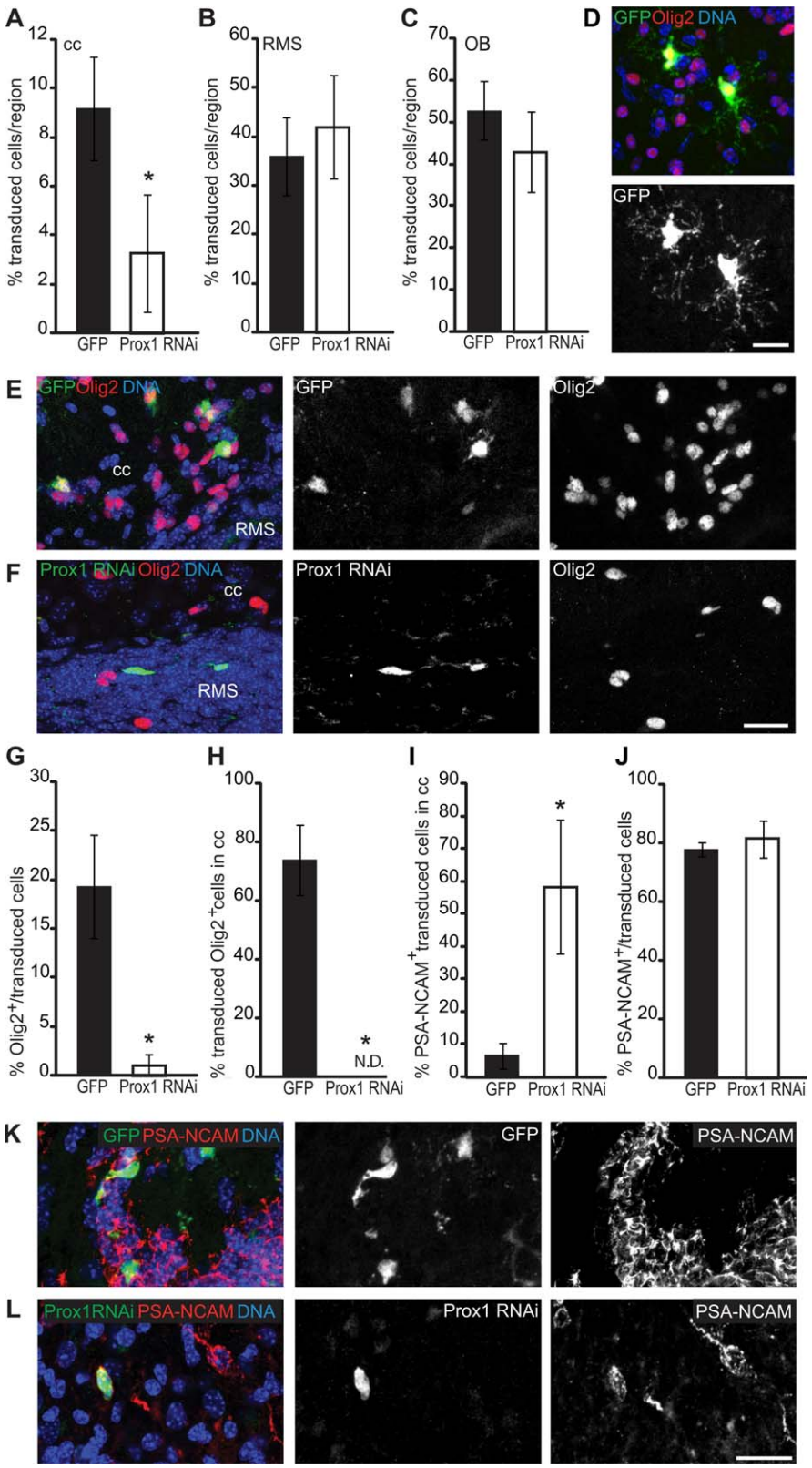

Figure 6.

between control and Prox1 knockdown, indicating that the per se ability of migration within Prox1 deficient cells itself was not affected (Fig. 6B, 6C). Furthermore, we analyzed the number of cells that had differentiated into oligodendrocytes by Olig2 expression. Under control conditions around 20% of all transduced cells were Olig2-positive while only less than 1% of the cells were found positive for Olig2 following Prox1 knockdown (control vs. Prox1-RNAi, $p = .02$) showing that cells lacking Prox1 possess impaired abilities to differentiate into oligodendrocytes (Fig. 6G).

Additionally, we specifically investigated the transduced cells within the cc. In control animals, around 75% of transduced cells expressed Olig2 (Fig. 6E, 6H). These cells typically showed a morphology of oligodendrocytic cells at least in a premyelination state as verified by ubiquitous GFP expression (Fig. 6D). However, no transduced cells that were also Olig2-positive were detectable within the cc following Prox1 knockdown (Fig. 6F, 6H, control vs. Prox1-RNAi, $p = .002$). Interestingly, despite the fact that only very few cells migrated into the cc following Prox1 knockdown, these cells were still positive for PSA-NCAM while their counterparts within the control animals did no longer show PSA-NCAM expression (control vs. Prox1-RNAi, $p = .05$) suggesting that cells lacking Prox1 no longer migrate into the cc and show diminished potential for oligodendrocyte differentiation (Fig. 6I, 6K, 6L). However, there was no difference in the overall number of transduced cells that expressed PSA-NCAM indicating that the effect of the loss of Prox1 is restricted to the generation of oligodendrocytes for the cc (Fig. 6J). As the total amount of transduced cells is not strongly differing between the control and Prox1-RNAi, we also conclude that a Prox1 knockdown has no major effect on proliferation or survival of neural progenitor cells. These data further support the notion that although Prox1 is transiently expressed in the neuronal lineage, it is not required for neurogenesis to proceed.

Prox1 Interacts with the Enhancer and Promoter Regions of *olig2* and *notch1* in cc Cells and Promotes Their Expression

Next, we were interested in the molecular mechanism used by Prox1 to promote oligodendrogenesis in neural stem cells derived from the SVZ. Recently, it was published that Prox1 directly interacts with the *notch1* promoter region within the developing spinal cord of mice and chick to promote neuronal differentiation [35]. To analyze whether this interaction could also be important for oligodendrocyte differentiation, we performed chromatin immunoprecipitation (ChIP) assays from freshly isolated cc cells derived from adult mice (Fig. 7). ChIP assays were performed using primers for the previously published interaction site of Prox1 at the *notch1* promoter as well as primers for the open reading frame (negative control). Since Olig2 has been shown to be involved in oligodendrogenesis [18, 36, 37] additional primers were designed for putative binding sites of Prox1 at the *olig2* gene [promoter region, K23 enhancer region, 3′-UTR (3′-Untranslated region of *olig2*) as negative control] (Fig. 7A, 7C). Indeed, ChIP analysis revealed that Prox1 directly interacts with the *olig2* promoter as well as the K23 enhancer (IgG vs. *olig2* promoter 1.5 ± 0.3 fold increase, $p = .02$; IgG vs. K23 Enhancer 2.1 ± 0.3 fold increase, $p < .001$) (Fig. 7B). Moreover, Prox1 also interacts with the *notch1* promoter region in cc cells (IgG vs. *notch1* promoter 4.6 ± 1.0 fold increase, $p = .001$) (Fig. 7D). To further elucidate whether Prox1 has an inhibiting or promoting function on *notch1* and *olig2* expression, luciferase assays were performed. Therefore, constructs where expression of the Firefly luciferase is under the control of the confirmed Prox1 binding sites of *notch1* and *olig2* were used (Fig. 7E, 7G). For both, *notch1* and *olig2*, expression of Prox1 resulted in a significant upregulation of the luciferase signal (Fig. 7F, control vs. Olig2 promoter 1.4 ± 0.1 fold increase, $p = .03$; control vs. Olig2 K23 enhancer 6.1 ± 0.3, $p = .03$; Fig. 7H control vs. Notch1 promoter 1.5 ± 0.3 fold increase, $p = .003$), suggesting that Prox1 promotes the expression of *notch1* as well as *olig2*.

Discussion

This study uncovers a novel function of the homeobox transcription regulator Prox1 in regulating adult oligodendrogenesis. Within the SVZ radial glia-like cells (type B cells) serve as adult neural stem cells that give rise to transiently amplifying type C cells. In turn, these cells proliferate and produce type A cells. Type A cells represent the migrating neuroblasts that were shown to differentiate into neurons for the OB. However, also oligodendrocytes are generated from SVZ NSCs but it has long been debated whether neurons and oligodendrocytes originate from the same neural stem cell type [6]. Data

Figure 6. Prox1 knockdown in SVZ progenitor cells results in a loss of adult generated oligodendrocytes for the corpus callosum in vivo but does not affect the number of newly generated cells within the RMS and OB. Retroviruses carrying a GPF or Prox1-RNAi-GFP expression cassette were injected into the SVZ of adult mice and animals were sacrificed 8 days following. Sagittal brain sections were stained for GFP (green) and Olig2 or PSA-NCAM (red) and GFP-positive cells were quantified and analyzed for expression of the respective marker. **(A)**: Transduced cells were quantified within the cc and a significant reduction was observed in animals subjected to Prox1 knockdown. **(B, C)**: Quantification of transduced cells in the RMS or OB revealed no differences in the number of cells following Prox1 knockdown compared to control transduction. **(D, E)**: In GFP virus transduced animals, GFP-positive cells that expressed Olig2 and resembling oligodendrocytic morphology were found within the cc. **(F)**: Following knockdown of Prox1, transduced cells were virtually absent from the cc. **(G)**: An overall reduction in the number of transduced cells within all brain regions analyzed that expressed Olig2 was observed; **(H)** within the cc no transduced cells that also expressed Olig2 were found. **(I-L)**: Quantification of transduced cells expressing PSA-NCAM. (J): No difference in the overall number of transduced cells that expressed PSA-NCAM between GFP and Prox1 knockdown was detected. (I): Following Prox1 knockdown significantly more cells within the cc remained PSA-NCAM expression compared to control cells. For quantification within the cc mice with no transduced cells in this region were excluded. (K, L): Representative images of sagittal brain sections analyzed by immunohistochemistry for GFP and PSA-NCAM expression. (K): GFP transduced cells in the cc did not express PSA-NCAM. (L): Following Prox1 knockdown, only few transduced cells were detected within the cc and these cells remained in PSA-NCAM expression. *$p = .004$ (A), *$p = .02$ (G), *$p = .002$ (H). Data are represented as mean ± SEM. Scale bars: (D) 20 μm, (E, F) 25 μm, (K,L) 20 μm. Abbreviations: cc, corpus callosum; GFP, green fluorescent protein; N.D., not detected; OB, olfactory bulb; PSA-NCAM, poly-sialated neural cell adhesion molecule; RMS, rostral migratory stream; SVZ, subventricular zone.

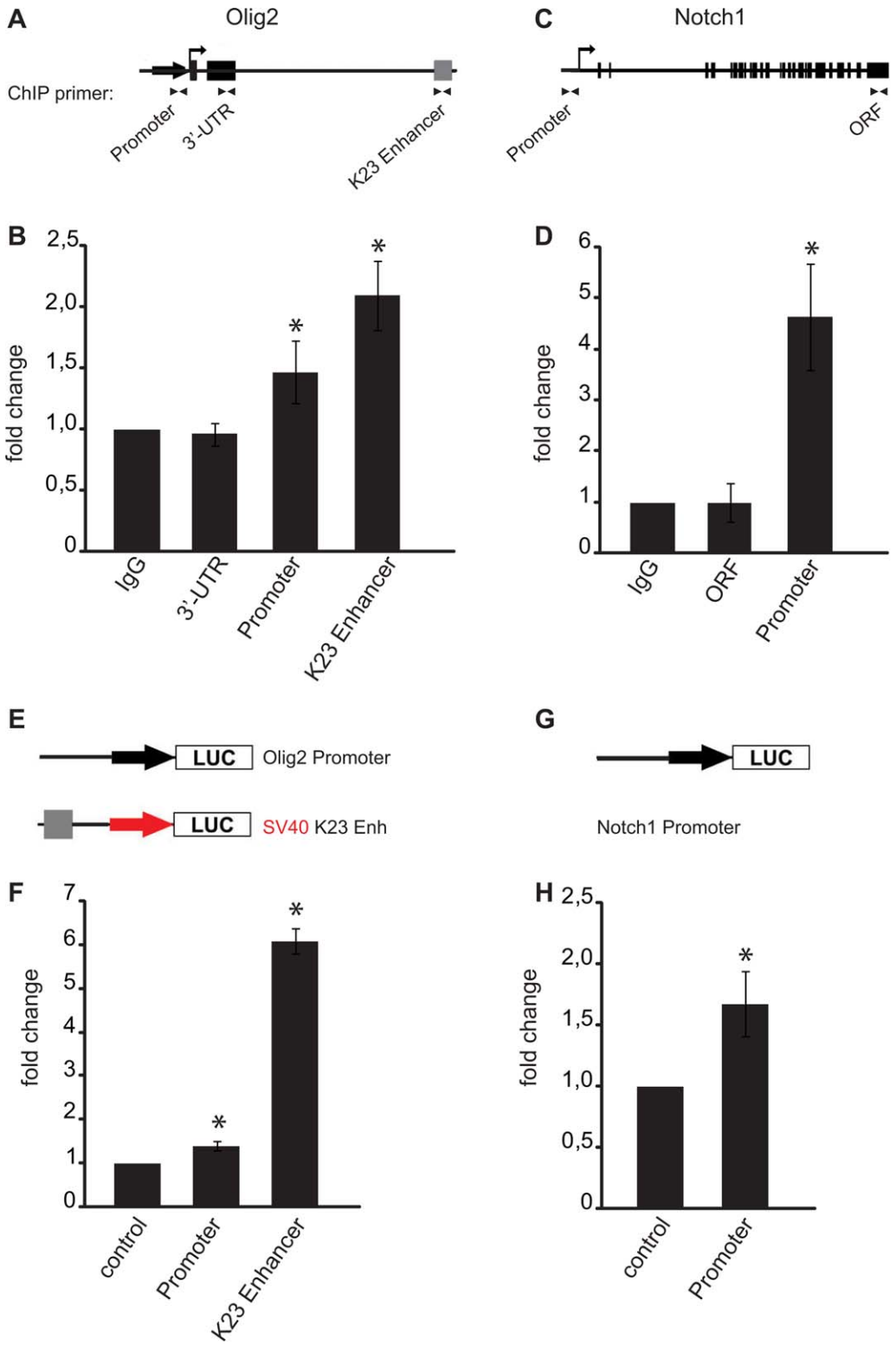

Figure 7. Prox1 interacts with the Enhancer and Promoter regions of *olig2* and *notch1* in corpus callosum cells and promotes their expression. **(A-D)**: ChIP assays were performed using anti-Prox1 antibody in chromatin prepared from cells isolated from the cc of adult mice. ChIP primer pairs were designed to amplify the corresponding DNA sequences for the proximal promoter region of the 1.1 Kb promoter, the 3′-UTR and the K23 enhancer of *olig2* (A) and the promoter region and ORF of *notch1* (C). Real-time Polymerase chain reaction (PCR) analysis revealed a significant increase in PCR products from the promoter and K23 enhancer regions of *olig2* (B) as well as the promoter region of *notch1* (D), while no increase was observed for the respective negative controls of *olig2* (3′-UTR) and *notch1* (ORF). **(E-H)**: Luciferase assays were performed using constructs carrying the Prox1 binding sites of *olig2* or *notch1* and the luciferase gene. Data are represented as mean ± SEM. $*p \leq .03$. Abbreviations: ORF, open reading frame; UTR, untranslated region.

from Menn et al. (2006) clearly demonstrated that SVZ type B cells are the primary source of oligodendrocytes. This finding has been followed up by several other studies addressing the dynamics of this process, the function of transcription factors like Olig1 as well as the contribution to remyelination [38–40].

Why some NSCs give rise to neurons, whereas others specify oligodendrocytes has been an open issue. Timed expression of certain transcription factors that induce one or the other lineage could be a possible mechanism for achieving lineage specification. Accordingly, we show here that the transcription factor Prox1 specifies oligodendrocyte identity in adult neural progenitor cells of the SVZ. However, expression of Prox1 commences during the transition of type C cells to type A cells. Therefore, the decision whether these progenitor cells become neurons or oligodendrocytes could either be made at the transition from type C to type A cells, or additionally other factors might be involved in initial steps toward oligodendrocyte specification while Prox1 is involved in later processes of oligodendrocyte identity determination. In line with the latter, our study shows that up to 55% of Prox1-positive cells in the SVZ express PSA-NCAM and that Prox1 expression decreases in these cells along their migration toward the OB. Therefore, Prox1 seems to be only transiently expressed in the neurogenic lineage of the SVZ while Prox1 expression remains in cells generated for the cc to drive oligodendrocyte differentiation. These results are further supported by our observation that in primary SVZ cultures Prox1 is downregulated following neuronal maturation while its expression remains high within the oligodendrogliogenic clones.

In summary, we found that Prox1 acts as a cell fate determinant for the oligodendrocyte lineage in neural progenitor cells of the adult SVZ. As Prox1 is expressed even before other oligodendrocyte marker become detectable, we conclude that it plays a role very early in the differentiation process. To date, we do not have evidence that Prox1 plays a role during the oligodendrocyte maturation process or for oligodendrocyte survival. However, such conclusion would require advanced models, like conditional or inducible knockout mouse models.

Previous work indicated that neural stem cells in the SVZ are organized in a mosaic pattern and that those cells in neonatal as well as in the adult brain inherit intrinsic information that restricts them in the type of neuron they can develop into [28]. Here, we show that Prox1 expression is not only important for oligodendrocyte differentiation of neural stem cells but also that Prox1 expressing cells of the SVZ are predominantly located in the dorsal area of the LV wall along the anterior-posterior axis, an area that was previously shown to generate oligodendrocytes for the cc [29, 41, 42]. Hence, there could be a direct correlation between cell position, Prox1 expression, and specification for an oligodendrocyte cell identity.

Interestingly, a recent publication by Kaltezioti et al. (2010) showed that Prox1 directly interacts with the *notch1* promoter region within the developing spinal cord to promote neuronal differentiation. As a down-stream target of pro-neural genes Prox1 seems to act as a repressor of *notch1* expression resulting in cell cycle exit and neurogenesis in this context [35]. We show here that Prox1 directly interacts with the *notch1* promoter region in cells isolated from the adult cc to promote its expression. We, thus, hypothesize that this mechanism could be involved in oligodendrogenic differentiation of these cells. In line with this, it has been demonstrated previously that non-classical Notch ligands like F3 and NB-3, belonging to the F3/contactin family, also activate Notch receptors on (NPCs) Neural Progenitor Cell and (OPCs) Oligodendrocyte Precursor Cell and that this activation, independent from Hes1 but mediated through Deltex1, results in oligodendrocyte differentiation and upregulation of myelin-related proteins [43, 44]. It is, therefore, conceivable that Prox1, as a transcription regulator, promotes or inhibits *notch1* expression depending on, yet to be identified, other intrinsic factors or cues from the local environment. Additionally, we demonstrate that Prox1 not only upregulates *notch1* expression but also directly interacts with the promoter and enhancer region of *olig2*, thereby promoting its expression. Olig2 was previously shown to exhibit binary functional characteristics during development as well as in the postnatal brain where it not only promotes proliferation of rapidly cycling transiently amplifying type C cells of the SVZ but also the terminal differentiation of myelinating oligodendrocytes that arise from these cells [7, 45, 46]. Furthermore, a study from Hack et al. (2005) illustrated that overexpression of Olig2 in SVZ neural precursor cells caused these cells to leave the RMS and to migrate into the cc supporting the role of Olig2 in oligodendrocyte differentiation of progenitor cells from the SVZ. Here, we now show a novel function of Prox1 in driving the expression of *olig2* in neural progenitor cells from the SVZ to promote oligodendrogenesis for the cc. Moreover, this process seems to be specific for SVZ-derived cells where Prox1 is exclusively expressed while, in contrast to these cells Prox1 expression is absent from local proliferating OPCs. However, with the here utilized experimental setup we cannot fully exclude that a specific local OPC subtype, with a very slow cell cycle time, might be missed. Such a cell type might be Prox1-positive.

Conclusion

Based on our here presented data we conclude that continuous expression of Prox1 acts as a cell fate determinant for the oligodendrocyte lineage in neural progenitor cells of the adult subventricular zone. Since SVZ-derived oligodendrocytes could provide a powerful source of cells for brain repair following demyelinating lesions [39], Prox1 could be a new promising target for cell replacement strategies.

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Author Contributions

E.C.B.: conception and design, acquisition of data, data analysis and interpretation, manuscript writing; L.G.C., S.V., M.A.P., M.v.C., A.S., J.B., F.O., B.B., and S.S.: acquisition of data, data analysis and interpretation, manuscript writing; G.E. and A.d.S.: target prediction, data analysis and interpretation, manuscript writing; P.K.P., F.O., B.B., M.S., and J.C.S.: conception and design of experiments, data analysis and interpretation, manuscript writing.

Disclosure of Potenial Conflicts of Interest

The authors indicate no potential conflicts of interest.

References

1 Bunk EC, Stelzer S, Hermann S et al. Cellular organization of adult neurogenesis in the Common Marmoset. Aging Cell 2011;10:28–38.

2 Curtis MA, Kam M, Nannmark U et al. Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. Science 2007;315:1243–1249.

3 Zhao C, Deng W, Gage FH. Mechanisms and functional implications of adult neurogenesis. Cell 2008;132:645–660.

4 Ertaylan G, Okawa S, Schwamborn JC et al. Gene regulatory network analysis reveals differences in site-specific cell fate determination in mammalian brain. Front Cell Neurosci 2014;8:437.

5 Lois C, Alvarez-Buylla A. Long-distance neuronal migration in the adult mammalian brain. Science 1994;264:1145–1148.

6 Ming GL, Song H. Adult neurogenesis in the mammalian brain: Significant answers and significant questions. Neuron 2011;70:687–702.

7 Menn B, Garcia-Verdugo JM, Yaschine C et al. Origin of oligodendrocytes in the subventricular zone of the adult brain. J Neurosci 2006;26:7907–7918.

8 Spassky N, Heydon K, Mangatal A et al. Sonic hedgehog-dependent emergence of oligodendrocytes in the telencephalon: Evidence for a source of oligodendrocytes in the olfactory bulb that is independent of PDGFRalpha signaling. Development 2001;128:4993–5004.

9 Tekki-Kessaris N, Woodruff R, Hall AC et al. Hedgehog-dependent oligodendrocyte lineage specification in the telencephalon. Development 2001;128:2545–2554.

10 Touahri Y, Escalas N, Benazeraf B et al. Sulfatase 1 promotes the motor neuron-to-oligodendrocyte fate switch by activating Shh signaling in Olig2 progenitors of the embryonic ventral spinal cord. J Neurosci 2012;32:18018–18034.

11 Kessaris N, Fogarty M, Iannarelli P et al. Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage. Nat Neurosci 2006;9:173–179.

12 Richardson WD, Kessaris N, Pringle N. Oligodendrocyte wars. Nat Rev Neurosci 2006;7:11–18.

13 Thomas JL, Spassky N, Perez Villegas EM et al. Spatiotemporal development of oligodendrocytes in the embryonic brain. J Neurosci Res 2000;59:471–476.

14 Wang J, Pol SU, Haberman AK et al. Transcription factor induction of human oligodendrocyte progenitor fate and differentiation. Proc Natl Acad Sci USA 2014;111:E2885–E2894.

15 Vue TY, Kim EJ, Parras CM et al. Ascl1 controls the number and distribution of astrocytes and oligodendrocytes in the gray matter and white matter of the spinal cord. Development 2014;141:3721–3731.

16 Young KM, Psachoulia K, Tripathi RB et al. Oligodendrocyte dynamics in the healthy adult CNS: Evidence for myelin remodeling. Neuron 2013;77:873–885.

17 Dawson MR, Polito A, Levine JM et al. NG2-expressing glial progenitor cells: An abundant and widespread population of cycling cells in the adult rat CNS. Mol Cell Neurosci 2003;24:476–488.

18 Fancy SP, Zhao C, Franklin RJ. Increased expression of Nkx2.2 and Olig2 identifies reactive oligodendrocyte progenitor cells responding to demyelination in the adult CNS. Mol Cell Neurosci 2004;27:247–254.

19 Gonzalez-Perez O, Alvarez-Buylla A. Oligodendrogenesis in the subventricular zone and the role of epidermal growth factor. Brain Res Rev 2011;67:147–156.

20 Nait-Oumesmar B, Picard-Riera N, Kerninon C et al. Activation of the subventricular zone in multiple sclerosis: Evidence for early glial progenitors. Proc Natl Acad Sci U S A 2007;104:4694–4699.

21 Misra K, Gui H, Matise MP. Prox1 regulates a transitory state for interneuron neurogenesis in the spinal cord. Dev Dyn 2008;237:393–402.

22 Risebro CA, Searles RG, Melville AA et al. Prox1 maintains muscle structure and growth in the developing heart. Development 2009;136:495–505.

23 Sosa-Pineda B, Wigle JT, Oliver G. Hepatocyte migration during liver development requires Prox1. Nat Genet 2000;25:254–255.

24 Lavado A, Oliver G. Prox1 expression patterns in the developing and adult murine brain. Dev Dyn 2007;236:518–524.

25 Miyoshi G, Young A, Petros T et al. Prox1 regulates the subtype-specific development of caudal ganglionic eminence-derived GABAergic cortical interneurons. J Neurosci 2015;35:12869–12889.

26 Karalay O, Doberauer K, Vadodaria KC et al. Prospero-related homeobox 1 gene (Prox1) is regulated by canonical Wnt signaling and has a stage-specific role in adult hippocampal neurogenesis. Proc Natl Acad Sci U S A 2011;108:5807–5812.

27 Lavado A, Lagutin OV, Chow LM et al. Prox1 is required for granule cell maturation and intermediate progenitor maintenance during brain neurogenesis. PLoS Biol 2010;8:e1000460.

28 Merkle FT, Mirzadeh Z, Alvarez-Buylla A. Mosaic organization of neural stem cells in the adult brain. Science 2007;317:381–384.

29 Ortega F, Gascon S, Masserdotti G et al. Oligodendrogliogenic and neurogenic adult subependymal zone neural stem cells constitute distinct lineages and exhibit differential responsiveness to Wnt signalling. Nat Cell Biol 2013;15:602–613.

30 Conti L, Cattaneo E. Neural stem cell systems: Physiological players or in vitro entities? Nat Rev Neurosci 2010;11:176–187.

31 Besson A, Dowdy SF, Roberts JM. CDK inhibitors: Cell cycle regulators and beyond. Dev Cell 2008;14:159–169.

32 Cmielova J, Rezacova M. p21Cip1/Waf1 protein and its function based on a subcellular localization [corrected. J Cell Biochem 2011;112:3502–3506.

33 Jakoby MJ, Weinl C, Pusch S et al. Analysis of the subcellular localization, function, and proteolytic control of the Arabidopsis cyclin-dependent kinase inhibitor ICK1/KRP1. Plant Physiol 2006;141:1293–1305.

34 Jablonska B, Aguirre A, Raymond M et al. Chordin-induced lineage plasticity of adult SVZ neuroblasts after demyelination. Nat Neurosci 2010;13:541–550.

35 Kaltezioti V, Kouroupi G, Oikonomaki M et al. Prox1 regulates the notch1-mediated inhibition of neurogenesis. PLoS Biol 2010;8:e1000565.

36 Hack MA, Saghatelyan A, de Chevigny A et al. Neuronal fate determinants of adult olfactory bulb neurogenesis. Nat Neurosci 2005;8:865–872.

37 Sun T, Hafler BP, Kaing S et al. Evidence for motoneuron lineage-specific regulation of Olig2 in the vertebrate neural tube. Dev Biol 2006;292:152–164.

38 Dai J, Bercury KK, Ahrendsen JT et al. Olig1 function is required for oligodendrocyte differentiation in the mouse brain. J Neurosci 2015;35:4386–4402.

39 Xing YL, Roth PT, Stratton JA et al. Adult neural precursor cells from the subventricular zone contribute significantly to oligodendrocyte regeneration and remyelination. J Neurosci 2014;34:14128–14146.

40 Brousse B, Magalon K, Durbec P et al. Region and dynamic specificities of adult neural stem cells and oligodendrocyte precursors in myelin regeneration in the mouse brain. Biol Open 2015;4:980–992.

41 Azim K, Raineteau O, Butt AM. Intraventricular injection of FGF-2 promotes generation of oligodendrocyte-lineage cells in the postnatal and adult forebrain. Glia 2012;60:1977–1990.

42 Relucio J, Menezes MJ, Miyagoe-Suzuki Y et al. Laminin regulates postnatal oligodendrocyte production by promoting oligodendrocyte progenitor survival in the subventricular zone. Glia 2012;60:1451–1467.

43 Cui XY, Hu QD, Tekaya M et al. NB-3/Notch1 pathway via Deltex1 promotes neural progenitor cell differentiation into oligodendrocytes. J Biol Chem 2004;279:25858–25865.
44 Hu QD, Ang BT, Karsak M et al. F3/contactin acts as a functional ligand for notch during oligodendrocyte maturation. Cell 2003;115:163–175.
45 Lu QR, Sun T, Zhu Z et al. Common developmental requirement for Olig function indicates a motor neuron/oligodendrocyte connection. Cell 2002;109:75–86.
46 Sun Y, Meijer DH, Alberta JA et al. Phosphorylation state of Olig2 regulates proliferation of neural progenitors. Neuron 2011;69:906–917.

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