Transcription factor induction of human oligodendrocyte progenitor fate and differentiation

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Human oligodendrocyte progenitor cell (OPC) specification and differentiation occurs slowly and limits the potential for cell-based treatment of demyelinating disease. In this study, using FACS-based isolation and microarray analysis, we identified a set of transcription factors expressed by human primary CD140a+ O4+ OPCs relative to CD133+ CD140a- neural stem/progenitor cells (NPCs). Among these, lentiviral overexpression of transcription factors ASCL1, SOX10, and NKX2.2 in NPCs was sufficient to induce Sox10 enhancer activity, OPC mRNA, and protein expression consistent with OPC fate; however, unlike ASCL1 and NKX2.2, only the transcriptome of SOX10-infected NPCs was induced to a human OPC gene expression signature. Furthermore, only SOX10 promoted oligodendrocyte commitment, and did so at quantitatively equivalent levels to native OPCs. In xenografts of shiverer/rag2 animals, SOX10 increased the rate of mature oligodendrocyte differentiation and axon ensheathment. Thus, SOX10 appears to be the principle and rate-limiting regulator of myelinogenic fate from human NPCs.

In this study, we sought to identify and characterize the rate-limiting transcription factors (TFs) that govern human OPC fate. Although several TFs are known to be necessary for oligodendrocyte fate and differentiation (reviewed in ref. 13), much less is known about those that act during OPC specification. Given the species differences in OPC gene expression (14), it is likely that the role of these TFs is subtly different in human cells. To select instructive TFs in an unbiased manner, we performed microarray analysis on antigenically defined human progenitors (15, 16). We compared the transcriptional profile of CD140a+ and O4-defined OPCs with that of CD133+ CD140a- NPCs. Because CD140a-depleted cells do not readily undergo oligodendrocyte differentiation, but likely represent their immediate developmental precursor, we induced the expression of potentially instructive OPC TFs in human NPCs by lentiviral overexpression. We found that although several TFs were capable of driving the expression of OPC-specific enhancer elements and individual genes, only SOX10 induced genome-wide reprogramming to resemble human CD140a-defined OPCs. Furthermore, enforced SOX10 expression alone was sufficient to induce oligodendrocyte differentiation at equivalent levels to native OPCs in vitro and to enhance the rate of differentiation and myelinization of xenografted NPCs in hypomyelinating shiverer/rag2 mice.

Results

FACS-Array Identification of Human OPC-Induced and Specific TFs. To select TFs in an unbiased manner, we performed microarray analysis on FACS-isolated human progenitor populations. We directly compared CD133+ CD140a- neural progenitor cells with neural precursor cell | transplantation | reprogramming

Significance

Transplantation of human myelinogenic cells represents a realizable strategy for treatment of congenital and acquired demyelinating diseases. Although generation of undifferentiated neural stem and progenitors is feasible, the induction of myelinogenic cell fate remains a significant challenge. In this paper, we describe, to our knowledge, the first comprehensive study of transcription factor expression and function by purified neural and oligodendrocyte progenitors obtained directly from human brain tissue. We have identified those transcription factors capable of regulating oligodendrocyte progenitor fate and establish that among these, only SOX10 was capable of comprehensively inducing oligodendrocyte fate both in vitro and following transplantation into a model of human leukodystrophy. Thus, viral and pharmacologic approaches to increasing SOX10 expression likely will improve the outcome of human transplant therapy.

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committed OPCs defined by CD133⁺CD140a⁺ antigenicity (15) as well as with CD140a/O4-defined OPCs. We identified 12 TFs that were up-regulated during the specification of OPC fate and/or between O4⁺ oligodendrocyte-biased and bipotential CD140a/PDGFRα⁺ OPCs (Fig. S1). We confirmed the expression profiles of these TFs using quantitative RT-PCR (qPCR) (Fig. S2), and selected a set of eight TFs for further functional analysis. We also chose ASCL1 because it has been shown to be necessary and sufficient to induce immature oligodendrocyte fate from mouse neural progenitors (17).

**A Subset of OPC TFs Activate the Sox10-MCS5 Enhancer in Human NPCs.** We selected a species conserved SOX10 as an NPC marker (16), and can be used to identify human NPCs (18). At 24 h after isolation, NPCs were infected with a Sox10-MCS5:GFP reporter virus and a candidate TF or mCherry-expressing virus as a negative control. Matched CD140a⁺ NPCs were used as a positive control in this and all other experiments. GFP expression was first detected in CD140a⁺ cells after 2 d and increased in intensity thereafter. Mean GFP intensity was measured at 3–4 d by flow cytometry (n = 3–9 fetal samples) (Fig. 1). As expected, CD140a⁺ OPCs expressed high levels of GFP, >2.8 fold greater than that expressed by uninfected NPCs (P < 0.05).

We found that ASCL1, NKX2.2, and SOX10 were each capable of significantly inducing Sox10-MCS5 enhancer activity (P < 0.01, normalized to mCherry control). None of the other factors induced GFP expression. Apart from ASCL1, all other factors drove enhancer activity at significantly lower levels than CD140a⁺ OPCs (P < 0.05, Dunnett’s post hoc test). These data indicate that ASCL1, NKX2-2, SOX10, and, to a lesser extent, OLIG2 and SOX8, regulate Sox10 enhancer activity in human NPCs.

**Single TF Infection Can Induce Markers of OPC Fate.** We next asked whether enforced TF expression could lead to induction of OPC markers. Following infection, NPCs were maintained for 4–7 d before RNA extraction or immunocytochemistry (Fig. 2). For qPCR, we selected PDGFRA and CSPG4, encoding PDGFRA/CD140a and NG2 proteins, respectively. As expected, PDGFRA mRNA was highly enriched in CD140a⁺ OPCs, by 13.5-fold relative to mCherry-infected NPCs, whereas CSPG4 mRNA enrichment was 3.2-fold higher (n = 3–5 fetal preparations) (Fig. 24). Several of the candidate TFs induced CSPG4 mRNA, including ASCL1, NKX2.2, and SOX10 (P < 0.05, Dunnett’s post hoc test, repeated-measures one-way ANOVA). Interestingly, ASCL1 induced significantly higher expression of CSPG4 mRNA than native OPCs (P < 0.05, paired t test). In contrast, only ASCL1 was able to up-regulate PDGFRA mRNA (Fig. 2B). Several factors, including NKX2.2 and PRRX1, seemingly repressed PDGFRA expression. To control for the effect of media on induction of these markers, we performed parallel experiments in both PDGF/FGF- and EGF/FGF- containing media (n = 2 fetal preparations), and found that gene expression changes were similar regardless of growth conditions.

We examined expression of antigenic markers of OPC fate using NG2- and A2B5-specific antibodies (Fig. 2 C–L). NPCs were infected and maintained in culture for 7 d in PDGF-AA/FGF supplemented media. CD140a⁺ OPCs maintained in the same condition were found to express high levels of both A2B5 and NG2. In control mCherry-infected NPCs, only 2.6 ± 0.5% expressed A2B5 (n = 3 fetal samples). In contrast, OLIG2, PRRX1, and SOX10 overexpression significantly induced A2B5 in 23–64% of NPCs (P < 0.05, Dunnett’s post hoc test, one-way ANOVA). Unlike A2B5, NG2 expression was not induced following OLIG2 overexpression, but was significantly induced in SOX10-, NKX2.2-, and PRRX1-infected cells. SOX10 consistently induced >60% of the infected NPCs to express both NG2 and A2B5.

Interestingly, the morphology of SOX10-infected NG2⁺ cells was altered such that several exhibited a flatter multipolar morphology compared with the bipolar appearance of native CD140a⁺ OPCs. ASCL1-infected NPCs rapidly underwent changes in cell morphology, becoming more fusiform and often with an enlarged bipolar appearance. We examined the expression of NG2 and A2B5 at 4 d and found that ASCL1 overexpression increased the abundance of both markers relative to mCherry-infected cells (Fig. 2 K and L). Taken together, these data indicate that ASCL1 and SOX10 have a strong effect on Sox10 enhancer, OPC marker mRNA, and protein expression; NKX2.2, OLIG2, and PRRX1 have a weaker effect on a subset of these markers; and only ASCL1 is capable of inducing PDGFRA mRNA.

![Fig. 1. OPC-specific TFs selectively activate Sox10-MCS5 enhancer. Human CD133⁺CD140a⁺ NPCs were coinfected with Sox10-MCS5:GFP reporter virus and individual OPC-specific TFs or mCherry control virus following isolation. (A–C) In contrast to control NPCs (mCherry), ASCL1-infected NPCs and OPCs contained a high proportion of cells expressing GFP at high levels (4 d in vitro). (D–F) Flow cytometry analysis of GFP expression, GFP<sub>high</sub>% (1,000 events shown). (G) Quantification of mean GFP intensity relative to matched mCherry control (mean ± SEM; n = 4–9 fetal samples). ** Indicates a significant increase in GFP expression; P < 0.01. (Scale bar: 100 μm.)](https://www.pnas.org/cgi/doi/10.1073/pnas.1408295111)
SOX10 Expression Induces an OPC-Like Gene Expression Signature.

We next sought to determine whether TF overexpression would regulate OPC-specific gene expression on a genome-wide scale. We performed Illumina microarray analysis for ASCL1-, SOX10-, and NKX2.2-infected NPCs and compared their expression with that in matched mCherry controls ($n = 3$–5 fetal brain preparations). Gene expression of TF-infected NPCs and uninfected OPCs is shown relative to mCherry-infected controls (log-twofold change, mean ± SEM; GAPDH-normalized). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, Dunnett’s posttest (one-way ANOVA). (A–J) Infected NPCs were immunostained with OPC markers A2B5 (C–F) and NG2 (G–J) and nuclei counterstained with DAPI (blue). Matching cultures were fixed at 4 d and 7 d. (K and L) Quantification of A2B5% (K) and NG2% (L) (mean ± SEM, $n = 3$–5). * $P < 0.05$; ** $P < 0.01$. (Scale bar: 50 μm.)

**Fig. 2.** Induction of OPC-specific genes by individual factors. Sorted CD133$^+$CD140a$^-$ NPCs infected with lentivirus were analyzed at 7 d by qPCR and immunocytochemistry. (A and B) qPCR analysis of OPC-specific genes CSPG4 and PDGFRA ($n = 3$–5 fetal brain preparations). Gene expression of TF-infected NPCs and uninfected OPCs is shown relative to mCherry-infected controls (log-twofold change, mean ± SEM; GAPDH-normalized). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, Dunnett’s posttest (one-way ANOVA). (C–J) Infected NPCs were immunostained with OPC markers A2B5 (C–F) and NG2 (G–J) and nuclei counterstained with DAPI (blue). Matching cultures were fixed at 4 d and 7 d. (K and L) Quantification of A2B5% (K) and NG2% (L) (mean ± SEM, $n = 3$–5). * $P < 0.05$; ** $P < 0.01$. (Scale bar: 50 μm.)

SOX10 Expression Induces an OPC-Like Gene Expression Signature.

We next sought to determine whether TF overexpression would regulate OPC-specific gene expression on a genome-wide scale. We performed Illumina microarray analysis for ASCL1-, SOX10-, and NKX2.2-infected NPCs and compared their expression with that in matched mCherry controls ($n = 2$ fetal preparations). We noted that the expression profiles of both ASCL1$^+$ and NKX2.2$^+$ infected cells were distinct from the profiles of mCherry-infected cells and also of each other, indicating divergent effects on gene expression (Fig. 3A).

Because the microarray probes for ASCL1, SOX10, and NKX2.2 are not located within the coding region of each gene, we examined the endogenous regulation of each factor (Fig. 3B).

As such, ASCL1 might induce its own expression while repressing NKX2.2 expression. SOX10 overexpression did not regulate these TFs. All three TFs reduced astrocyte-specific gene expression, including intermediate filament GFAP and glutamate transporter GLT-1 (SLC1A2). Neuronal-expressed transcripts, such as CD24 and doublecortin (DCX), were up-regulated after ASCL1 overexpression (by 9.6- and 15.9-fold, respectively). NKX2.2 similarly up-regulated CD24 (by 3.7-fold) and neuronal RNA-binding protein HuD (ELAVL4, by 7.3-fold). In contrast, SOX10 did not regulate neuronal markers. Both SOX10 and ASCL1 induced OPC-specific transcripts NG2 (CSPG4) and PDGFRA. SOX10 also induced the A2B5 synthetic enzyme ST8SIA1, as well as myelin transcripts PLP1 and UGT8 (Fig. 3C). Each TF also was capable of regulating a subset of specific OPC-expressed TFs, suggesting a hierarchy of TF activation in human NPCs (Fig. 3D).

Among ASCL1-induced genes, we found a very significant overrepresentation of genes involved in cell cycle and proliferation (topGO enrichment analysis, $P = 1.2 \times 10^{-25}$). In contrast, NKX2.2 up-regulated genes involved in regulation of synaptogenesis.
this enrichment was highly significant (Fig. 3). Among native OPCs relative to NPCs, and that are derived (Fig. 3).

We next used gene set enrichment analysis to include multiple transcription factors (TFs) following virus infection relative to mCherry. (E and F) ROC analysis of virally induced genes versus the profile of CD133+ CD140a+ OPCs relative to CD133+CD140a- NPCs. The definition of each line above the ‘line of identity’ (black diagonal) indicates enrichment of genes induced compared with mCherry infection. (F) GSEA examining relative enrichment of cell-type specific genes among virally induced gene signatures. Red indicates enrichment; blue, depletion.

SOX10-Induced OPCs Are Capable of Oligodendrocyte Commitment in Vitro. One hallmark of OPCs is their ability to expand in the presence of mitogens and undergo oligodendrocyte differentiation following mitogen withdrawal. To determine whether infected cells could differentiate as oligodendrocytes, we allowed infected NPCs serially grown in the presence of FGF-2/EGF (4 d) then FGF/PDGF-AA (6 d) to differentiate in the absence of mitogens at day 10 before fixation at 14 or 21 d (Fig. 4). On day 14, 21.6 ± 2.9% of infected cells differentiated into O4+ immature oligodendrocytes with typical branched morphology (n = 3–8 fetal preparations), and some matured into myelin sheath-forming oligodendrocytes (Fig. 4D, arrowhead). In comparison, few mCherry-infected control NPCs were O4+ (0.2 ± 0.2%). Likewise, individual overexpression of ASCL1, NKRX2-2, OLIG2, and PRX1 did not induce oligodendrocyte fate (Fig. 4). However, SOX10 infection induced a significant amount of immature oligodendrocytes (16.8 ± 3.2%), surprisingly close to the uninfected NPCs. Most SOX10-induced oligodendrocytes had the same morphology as those derived from primary OPCs (Fig. 4C).

Although not a specific marker of OPCs (20), OLIG2 is required for normal oligodendrocyte lineage differentiation (21) and is expressed by all oligodendrocyte lineage cells. As such, we colabeled cultures with OLIG2 to determine whether TF overexpression regulated OLIG2 expression (Fig. 4A–D and F). Similar to acutely isolated CD133+CD140a- NPCs (15), ~30% of mCherry-infected NPCs expressed OLIG2 protein. As expected, OLIG2 virus induced very strong OLIG2 protein expression (83 ± 9%; n = 3 fetal preparations). None of the other TFs up-regulated OLIG2. Interestingly, all SOX10-induced O4+ oligodendrocytes coexpressed OLIG2, suggesting that endogenous OLIG2 is
required for oligodendrocyte reprogramming. In contrast, ASCL1 overexpression induced a greater than fivefold increase in Tuj1+ neuron generation, along with concomitant near abolition of OLIG2 expression (1.3 ± 0.7%, n = 3; P < 0.05, one-way ANOVA) (Fig. 4F). Interestingly, combined ASCL1 and OLIG2 overexpression also gave rise to neuronal induction. Because OPC-expressed TFs may act to inhibit premature terminal differentiation, we asked whether transient overexpression...
may permit oligodendrocyte differentiation following mitogen withdrawal. Using a doxycycline-inducible lentiviral vector, we overexpressed candidate TFs, ASCL1, PRRX1, and SOX10 for 2 or 4 d in human NPCs. As with constitutive overexpression, transient overexpression was sufficient to induce NG2 expression at 7 d (Fig. S3); however, after mitogen removal, only SOX10 was capable of inducing O4+ oligodendrocytes. This finding suggests that the absence of oligodendrocyte differentiation following PRRX1 and ASCL1 is not limited by direct inhibition of differentiation.

To determine whether SOX10-infected NPCs are capable of maturation to MBP+ oligodendrocytes, we fixed matched cultures at 21 d (Fig. 4 G–J). As expected, primary CD140a+ OPCs generated numerous oligodendrocytes with MBP+ myelin sheaths (123 ± 19 cells/well, n = 3 fetal dissociates), whereas very few oligodendrocytes were observed in cultures of mCherry-infected NPCs (1.3 ± 1.3 cells/well). SOX10-infected NPCs generated a substantial number of MBP+ oligodendrocytes with a highly branched complex morphology. Using a HA-tagged SOX10 virus, we found that all MBP+ cells retained SOX10-HA expression at 21 d. Of the OPC-expressed TFs tested, only SOX10 was sufficient to induce rapid oligodendrocyte differentiation from primary human NPCs in vitro.

SOX10-Infected NPCs Are Proliferative Oligodendrocyte Progenitors in Vitro. Primary human OPCs differentiate in a context-specific manner and can be maintained as progenitors for several passages in vitro (22). To determine whether SOX10-infected NPCs could be maintained as proliferative progenitors, we grew mCherry- and SOX10-HA–tagged NPCs in the presence of PDGF-AA for three passages (>1 mo in vitro) and assessed oligodendrocyte differentiation at each passage (Fig. S4). No between-group differences in cell expansion were noted. A 24-h BrdU pulse before fixation revealed that the majority of SOX10-HA–expressing cells were proliferative while maintained in PDGF-AA and FGF-2. In addition, SOX10-HA+ cells colabeled with NG2 expression, whereas matched mCherry-infected cultures exhibited few NG2+ cells. Significantly, spontaneous differentiation of O4+ oligodendrocytes was not observed in cultures maintained in growth factors.

In an experiment to test whether SOX10-infected NPCs could still differentiate infected cells were pulsed for 24 h with BrdU and then grown in the absence of mitogens for 4 d and stained. At each passage, several SOX10-HA–infected cells differentiated as O4+ oligodendrocytes. Importantly, even at the third passage, we observed SOX10-HA+O4+BrdU+ cells, indicating that recently dividing SOX10-infected cells were able to undergo differentiation after mitogen withdrawal (Fig. S4). Taken together, these data suggest that SOX10 overexpression induces a proliferative progenitor that retains the capacity for oligodendrocyte differentiation similar to that of native OPCs.

SOX10 Overexpression Induces Rapid Myelination from Human NPCs. To determine the myelinating capacity of SOX10-infected NPCs, we injected 1 × 10^5 infected human NPCs into the corpus callosum of neonatal Shiverer2 mutant mice. NPCs were infected with either SOX10- or mCherry-expressing virus at 48 h before implantation. At 12 wk postimplantation, both mCherry- and SOX10-infected human NPCs exhibited a similar pattern of engraftment. Human cells (hNA+) were observed from olfactory bulbs to the caudal extent of fimbria, spanning >5 mm in all transplanted mice. Typically, transplanted human cells were located in white matter, such as corpus callosum, fimbria, and striatal white matter. Overall human cell engraftment was greater in callosum (550 ± 43 hNA+ cells/mm²) than in fimbria (140 ± 7 hNA+ cells/mm²).

We first assessed the effect of SOX10 overexpression on oligodendrocyte differentiation and maturation. At 12 wk post-transplantation, SOX10-infected NPCs underwent robust differentiation as CC1- and MBP-expressing oligodendrocytes. Importantly, SOX10 overexpression resulted in an almost twofold increase in the proportion of human hNA+ cells undergoing differentiation as hNA+CC1+ oligodendrocytes in the corpus callosum (mean ± SEM, 27 ± 4% vs. 14 ± 2%; P < 0.05, unpaired t test; n = 3) (Fig. 5 A and B). Although myelin basic protein (MBP) expression was produced by both engrafted groups (Fig. 5C), in the fimbria where individual MBP+ oligodendrocytes could be reliably counted, we found significantly more MBP+ oligodendrocytes derived from SOX10-infected NPCs compared with matched mCherry-infected cells (25 ± 2% vs. 14 ± 1%; n = 3 animals per group) (Fig. 5 D, E, and K).

In addition, we noted a profound difference in the intracellular distribution of MBP between mCherry and SOX10-infected cells in the corpus callosum (Fig. 5 F and G and Fig. S5). MBP was localized in the cell body of human oligodendrocytes derived from mCherry-infected NPCs, whereas MBP was exclusively localized to axonal segments in animals receiving SOX10-infected NPCs. Because differential localization of MBP has been associated with progressive myelination by human cells (3), we compared the efficiency of callosal axonal ensheathment by each cell type (5) (Fig. 5 H–J). SOX10-infected NPCs ensheathed >30% of axons in the corpus callosum (32 ± 1%: n = 3 animals), more than twice the proportion ensheathed by mCherry-infected cells. Importantly, the extent of ensheathment by SOX10-infected NPCs exceeded that of human iPSC-derived glial progenitors at 13 wk (10), and was equivalent to that of native fetal CD140a-defined OPCs at this time point (5). Taken together, these data indicate that oligodendrocyte differentiation and myelogenesis from primary NPCs is induced by enforced SOX10 expression, and that SOX10-induced neural stem cells produce myelin at a similar rate as native human OPCs.

SOX10-Infected NPCs Resemble Native OPCs after Engraftment in Shiverer Mice. Given that SOX10 overexpression significantly increased oligodendrocyte differentiation and myelination by human NPCs in vivo, we asked whether this effect is related to regulation of NPC homeostasis, increased OPC specification, and/or differentiation. Human nestin-positive NPC-expressing cells were found in the corpus callosum in both groups of animals at 12 wk (Fig. 6 A). Several nestin-positive cells were located surrounding the lateral ventricles and extended processes through the ependymal layer and as such morphologically resembled native neural stem cells (Fig. 6 A, Inset). Thus, transplanted NPCs persisted in the murine parenchyma after transplantation.

Importantly, we found no quantitative between-group difference in cell density (n = 3 per group, unpaired t test). The proportion of mCherry and SOX10-infected cells expressing markers of proliferation was also similar in the two groups. Among scored animals, the fraction of Ki67+hNA+ cells was 9.5 ± 0.6%, similar to that of primary CD140a+ OPCs (3). More importantly, SOX10-HA–expressing cells were still proliferative at 12 wk, expressing Ki67 and PCNA (Fig. 6 B). These data suggest that SOX10 overexpression does not induce differentiation at the cost of reduced cell engraftment, migration, and proliferation. At 8 wk posttransplantation, significant numbers of human NG2+ OPCs were found in the corpus callosum and fimbria of animals receiving SOX10-induced OPCs (Fig. S6). In contrast, few mCherry-infected NPCs expressed NG2. By 12 wk, human NG2+ cells pervaded the corpus callosum and forebrain white matter in both groups, and no obvious differences in OPC density were observed (Fig. 6 C). Examination of oligodendrocyte differentiation at 8 wk showed that significantly more SOX10-infected NPCs differentiated as CC1+hNA+-differentiated human oligodendrocytes compared with mCherry control (P < 0.05, t test) (Fig. 6 D–G). Furthermore, although very few MBP+
mature oligodendrocytes were observed in mCherry-infected NPC-engrafted animals at 8 wk, MBP+ cells were diffusely present throughout the corpus callosum and fimbria of all SOX10-transplanted mice (n = 3 per group) (Fig. S6 H–K). These data suggest that SOX10-infected NPCs resemble native OPCs in several respects, retaining mitotic competence and NG2+ expression for several months and progressively differentiating as MBP+ oligodendrocytes in vivo.

**SOX10 Overexpression Inhibits Astrocytic Commitment from NPCs.** Because human CD140a+ OPCs also differentiate into fibrous astrocytes following transplantation (5), we determined the proportion of infected human NPCs that expressed GFAP+ astrocytes at 12 wk (Fig. 6 D–F). Interestingly, SOX10 overexpression significantly impaired the production of GFAP+ astrocytes compared with mCherry-infected NPCs (quantified in the corpus callosum; 18 ± 5% in SOX10-infected vs. 40 ± 5% in mCherry-infected; unpaired t test, n = 3) (Fig. 6F). We hypothesized that the quantitative level of SOX10 expression in OPCs could influence oligodendrocyte differentiation. To directly test this hypothesis, we injected primary human CD140a+ OPCs with SOX10 or mCherry lentivirus and assessed oligodendrocyte and astrocyte differentiation at 4 d after infection (Fig. S7). Consistent with our in vivo data, enforced SOX10 expression induced O4+ oligodendrocyte differentiation at the cost of GFAP+ astrocytic commitment. Taken together, these data indicate that SOX10 overexpression is not only capable of inducing rapid OPC fate commitment, but also promotes myelinogenic oligodendrocyte differentiation from human NPCs.

**Discussion**

**Identification of OPC-Specific TFs.** In this study, we sought to identify TFs that may regulate human OPC fate during fetal development. We hypothesized that TF up-regulation of inductive factors would coincide with acquisition of OPC fate. We chose CD140a/PDGFA receptor antigen because it represents an early and specific marker of human OPCs (5). Through a combination with CD133, we were able to distinguish CD133+CD140a− NPCs from CD140a+ OPCs (15). Genomic analysis of CD140a+ OPCs relative to NPCs identified known OPC-specific TFs, including SOX10, Olig2, Nkx2.2, Myt1, Id2, and Hes5 (23). In contrast, our analysis did not identify TFs that specifically regulate oligodendrocyte differentiation (e.g., Myrf, Znf488, Nkx6.2). This approach allowed us to identify TFs expressed by OPCs at the time of specification. It is conceivable, however, that key TFs may be transiently up-regulated during transition to OPC fate, and that isolation of OPCs en masse would not be resolved.

In addition to known TFs, we identified several previously unidentified OPC-expressed TFs. In addition to fetal OPCs, Prrx1, a paired-type homeodomain factor, is also highly expressed in human adult OPCs (14) and rodent OPCs (19, 24). We found that Prrx1 overexpression induced OPC antigens A2B5 and NG2, suggesting that it may directly induce OPC fate; however, by itself, it was not sufficient to permit oligodendrocyte differentiation. Interestingly, ectopic Prrx1 is known to inhibit neuronal differentiation of adult murine NPCs and thereby promote self-renewal (25). Rfx4, a winged helix DNA-binding protein, is up-regulated in fetal OPCs compared with NPCs, and its expression is maintained in adult human OPCs (14). Interestingly, Rfx4 is highly expressed by both human and mouse astrocytes as well, suggesting a role in both glial subtypes (14, 19). Knockout of RFX4 down-regulates Wnt ligand and induces Id4 expression in early embryonic brain, implicating it in the specification and development of glia (26); however, RFX4 overexpression does not affect expression of OPC markers in human NPCs, and a precise role in glia remains to be determined. Both Pou2f1 (Oct-1) and Tcf12 likely act as accessory transcriptional regulators to Sox and class B bHLH proteins, respectively. Although widely expressed in development, the quantitative increase in Pou2f1 and Tcf12 mRNAs...
in CD140a+ OPCs suggests a role in OPC specification that was not revealed by overexpression.

**Induction of Human OPC and Oligodendrocyte Fate.** Because NPCs represent the direct developmental forerunners of OPCs, we hypothesized that overexpression of a single TF would direct OPC and oligodendrocyte fate. By defining the TF factor/s sufficient to induce fate, we anticipated that success would permit mechanistic analysis of TF activity in NPCs before the analysis of TF combinations. The induction of oligodendrocyte fate by individual TF overexpression has been studied in several model organisms, including zebrafish, chick, and mouse. It appears that multiple TFs can induce oligodendrocyte differentiation from neural precursors, including OLIG1/2, NKK2.2, ASCL1, SOX10, SOX17, and others (17, 27–34); however, the instructive role of individual factors toward oligodendrocyte fate has not been observed consistently across species and systems. For example, ASCL1 is capable of inducing rapid oligodendrocyte differentiation in cultured mouse neural stem/progenitors (17), but retroviral-mediated expression in embryonic mouse forebrain does not induce oligodendrocyte fate (32). In human fetal neurospheres, OLIG2 overexpression leads to increased expression of A2B5 that does not yield significant oligodendrocyte differentiation in *shiverer* mice (35). As such, the robust induction of OPC and oligodendrocyte fate from human NPCs by TF overexpression has not yet been achieved.

Because expression of individual markers such as A2B5, NG2, and OLIG2 is not restricted to OPCs, we sought to define OPC fate through a combination of marker expression, activity of known enhancer elements, genome-wide expression profiles, and functional characteristics, growth factor proliferation and oligodendrocyte differentiation. These results are summarized in Table S1. Of the factors tested, only SOX10 overexpression was capable of inducing a proliferative progenitor cell that rapidly differentiates into oligodendrocytes both in vitro and in vivo.

**Context-Specific Effects of TF Overexpression.** When induced to differentiate by mitogen withdrawal, SOX10-infected NPCs generated equivalent numbers of O4+ and MBP+ oligodendrocytes to native primary CD140a-sorted OPCs. Induced oligodendrocytes were restricted to the OLIG2-expression fraction of NPCs. This suggests that OLIG2 is necessary for OPC fate, as has been demonstrated in rodents (31, 36). Interestingly, coinfection with SOX10 and OLIG2 viruses did not result in a significant increase in O4+ oligodendrocyte differentiation, suggesting that OLIG2 is not the sole limiting factor for OPC fate induction by SOX10. Indeed, because both binding partners (37) and phosphorylation state (38) can regulate the function of OLIG2, overexpression of OLIG2 alone might not provide the correct cellular context for induction of OPC fate.

Likewise, we observed that whereas ASCL1 or PRRX1 overexpression was sufficient for some indicators of OPC fate (Table S1), oligodendrocyte differentiation was not observed from infected NPCs after growth factor removal. The principal effect of ASCL1 overexpression was induction of a neuronal-like morphology and βIII-tubulin expression. Interestingly, transient expression was also sufficient to induce NG2 expression, but did not lead to oligodendrocyte differentiation after growth factor removal. As observed with constitutive expression, transient ASCL1 overexpression induced neuronal-like differentiation. Although OLIG2 was repressed in ASCL1-infected NPCs, thereby possibly limiting differentiation, concurrent infection with ASCL1 and OLIG2 did not induce oligodendrocyte differentiation. This suggests that the absence of oligodendrocyte differentiation following ASCL1 or PRRX1 overexpression is related not to direct inhibition of differentiation, but rather to the lack of another factor (possibly SOX10) that limits the differentiation capacity of induced OPCs. Thus, additional work is needed to precisely define the combinations, timing, and posttranscriptional regulation of these factors to increase the efficiency of OPC fate.

Somatic cell reprogramming through various combinations of TF overexpression has been established as a novel means to induce transdifferentiation of various somatic cell types, typically fibroblasts, to neuronal and other lineages (39). Two recent studies have demonstrated this approach with rodent fibroblasts and induction of OPC-like and oligodendrocyte fate using a combination of Sox10, Olig2, and either Zfp546 or Nkx6.2 (40, 41); however, neither of these approaches has been...
successful thus far in human fibroblasts. Given the transcriptional differences between mouse and human OPCs (14), the specific factors sufficient to induce OPCs from human cells likely are different. Indeed, when we examined which genes were activated by SOX10 in human NPCs, we found no significant enrichment relative to known rodent target genes [receiver operating characteristic (ROC)-based area under the curve analysis] (30). These data suggest that the precise effect of each overexpressed TF may differ based on species and cellular environment.

In summary, we found that directly targeting SOX10 expression in human NPCs promoted OPC specification, oligodendrocyte differentiation, and myelination of transplanted human progenitor cells. These data suggest that SOX10 is one of the principle gatekeepers for oligodendrocyte lineage fate. Thus, small molecules or cell signaling cascades that induce SOX10 expression might be expected to enhance OPC specification and myelination by both endogenous and transplanted human cells. Because cells derived from pluripotent stem cells transition through the same developmental stages, we hypothesize that agents capable of inducing SOX10 expression and precocious myelination likely would be similarly effective and useful as adjuncts to iPSC-based approaches. Alternatively, by identifying the key targets of SOX10 that drive oligodendrocyte differentiation, we may be able to similarly promote differentiation and myelination in a variety of neurologic diseases without directly targeting SOX10.

Materials and Methods

Tissue Samples. Fetal brain samples at 15–22 wk gestational age were obtained from patients who consented to tissue use under protocols approved by the local Institutional Review Board. Dispersates were prepared as described previously (42) and cultured in serum-free medium (SFM) as detailed previously (5) with 10 ng/mL FGF2 (PeproTech).

Cytometry/FACS. Cytometry and sorting were performed with a BD FACSAria cell sorter, as described previously (15). For CD133/CD140a, cells were stained with CD140a-PE (BD Pharmingen) and CD133-APC (Miltenyi Biotech). Matched fluorescence minus-one controls were used to set gates following doublet discrimination.

qPCR Analyses. Immediately after CD133/CD140a FACS, RNA extraction, first-strand synthesis, and qPCR were performed, as described previously (16). Human primers for SYBR Green-based PCR and predesigned primer and Taqman probes were purchased from IDT and Invitrogen, respectively (Table S2). Samples were run in duplicate, and gene expression was calculated by ΔΔCT analysis using GAPDH as a reference gene. GAPDH was chosen following initial experiments that used 18S RNA as a reference gene. Because both reference genes showed very high correlation (R² = 0.91), we used GAPDH in all subsequent experiments.

To determine the effect of TF overexpression of OPC-specific gene expression, we plated CD133+CD140a− NPCs onto polystyrene/laminin substrate and infected them the next day with lentivirus. Cells were maintained in SFM with either 20 ng/mL EGF and 20 ng/mL PDDG-2 or 20 ng/mL PDGFAA and 5 ng/mL PDDG-2 before extraction and qPCR analysis 7 d later.

TF and Lentiviral Cloning. We cloned the coding regions of each identified factor into a lentiviral backbone derived from pTRIP-EF1α (43) (a gift from Abdel Benraiss, University of Rochester, Rochester, NY). In brief, human TF coding sequences (CDSs) were obtained either from existing plasmids or reverse-transcribed human CDNA and then PCR-cloned into pTRIP.1 TOP04 plasmid (Life Technologies). The CDS was then transferred into pTRIP-EF1α by replacing mCherry using unique restriction sites (Table S3). Lentiviruses were prepared as described previously (44). In brief, after triple transfection of HEK 293T cells with pTRIP and packaging plasmids plPVSVG (Life Technologies) and psPAX2 (AddGene), viral supernatant was collected at 48 and 72 h. Titration of virus was performed on matched mCherry-expressing virus using flow cytometry for mCherry fluorescence and directly compared with TF viruses using qPCR for the WPRE sequence (45). Confirmation of TF expression was performed after infection in 293T cells (Table S3). Lentiviruses were added to culture at 1 multiplicity of infection (MOI) for 24 h, followed by complete medium replacement unless stated otherwise.

Sox10-MC55:GFP Reporter. Following FACS, cells were plated at 5 × 10⁶/mL on polyornithine/laminin-coated plates in SFM with EGF/PFGF2 (20 ng/mL; PeproTech). On the next day, cells were infected with Sox10-MC55:GFP reporter lentivirus at 1 MOI (18). Virus carrying individual TFs was added to cell culture at 1 MOI 24 h after Sox10-MC55:GFP virus infection. Cytometry was performed 4 d thereafter. Cells were stained using FSC, SSC, and GFP expression was measured.

In Vitro Immunostaining. Following FACS, human CD133+CD140a− NPCs were plated at 5 × 10⁵/mL on polyornithine/laminin-coated 24-well plates in SFM supplemented with 20 ng/mL EGF/PFGF2. At 24 h later, cells were infected with lentivirus at 1 MOI. For assessment of OPC induction, cells were fixed with AzB5 (1:1, hybridoma supernatant; American Type Culture Collection) or fixed for NG2 (mouse IgG2a, 1:200; Millipore). For oligodendrocyte differentiation, cells were further maintained in SFM supplemented with 20 ng/mL PDGF and 5 ng/mL PFGF2 for 6 d, followed by 5 ng/mL NT-3 until day 14 or 21. Cells were immunostained for OL2/40 as described previously (16), or with MBP (rat IgG, 1:200; Abcam). Alexa Fluor 594, 647, and 488-conjugated goat secondary antibodies (Life Technologies) were used at 1:500 dilution. In all experiments, uninfected human CD140a− OPCs were maintained in the same conditions as positive controls. For cell counting, at least 200 live cells were counted from five randomly selected fields (20x) or >500 cells along the diameter of the well.

Microarray Analysis. Human CD133+CD140a− NPCs were infected with individual TFs or mCherry-expressing lentivirus at 24 h post-FACS. Infected cells were maintained in mitotic conditions containing EGF/PFGF for another 7 d before RNA extraction. To determine the expression profile of infected cells, we performed illumina microarray analysis (n = 2 fetal samples) and combined these data with the expression profile of CD133/CD140a sorted cells (n = 3 fetal samples) (15). RNA was amplified and Illumina HT-12v4 bead arrays analysis was performed using R/Bioconductor, as described previously (16). We identified genes as significantly regulated using a moderated t test statistic (P < 0.01) and further filtered these genes to examine only those regulated by two or more. Gene set enrichment analysis was performed (46) using a custom gene set collection compiled from human CD140a/04 (16), CD133/CD140a (15), and mouse immunopanned cells (19). ROC-based area under curve data and significance of gene set enrichment were calculated using roc.area (47). TF-infected cell populations were compared using roc.test (48).

Transplantation into shiverer/rag2 mice. All experiments using shiverer/rag2 mice (a gift of Dr. Steven A. Goldman, University of Rochester, Rochester, NY) (3) were performed according to protocols approved by the University at Buffalo’s Institutional Animal Care and Use Committee. If necessary, newborn pups were genotyped on the day of birth to identify homozygote shiverer mice. Human NPCs and OPCs were cultured for up to 1 wk in SFM containing EGF/FGF and PDGF/FGF, respectively, and frozen using ProFreeze (Lonza) before surgery. At 24 h after thawing, cells were infected with lentivirus at 1 MOI and then allowed to recover for 1–2 d before surgery. Cells were prepared for injection by resuspending cells in HBSS (1×) and then centrifuging at 1,000 × g. In brief, mice were anesthetized using isoflurane and 5 × 10⁴ cells were injected in each site, bilaterally, at a depth of 1.1 mm into the corpus callosum of postnatal day 2–3 pups. Cells were injected through pulled glass pipettes inserted directly through the skull into the presumptive target sites. Animals were killed and perfused with saline, followed by 4% paraformaldehyde, at 8 or 12 wk.

In Vivo Immunostaining. Cryopreserved coronal sections of mouse forebrain (16 μm) were cut, and brains were sampled every 160 μm. Immunohistochemistry was performed as described previously (5). Human cells were identified with mouse anti-human nuclei (mouse IgG1, 1:100, clone 233–1; Millipore), and myelin basic protein-expressing oligodendrocytes were labeled with MBP. Oligodendrocytes, human astrocytes, human OPCs, and human NPCs were recognized by CC1 (mouse IgG2b, 1:50; EMD Chemicals), hGFAP (mouse IgG1, 1:800; Covance), hNG2 (mouse IgG2a, 1:800; Millipore), and Nestin.

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(mouse IgG1, 1:1000; Millipore), respectively. Mouse neurofilament (NF) was stained with a 1:1 mixture of SM311 and SM312 (mouse IgG1, 1:800; Covance). Alexa Fluor secondary antibodies, goat anti-mouse 488, rat 594, and rabbit 647 (Life Technologies), were used at 1:500.

**Microscopy.** To quantify the proportion of MBP+ oligodendrocytes in the fimbria, we sampled four sections at random from every 160 μm with a 40× objective in each animal. Between 500 and 1,000 hNA+ MBP+ cells were counted for each animal. The proportion of mCherry- and HA-expressing MBP+ cells was assessed in a similar manner. For assessment of human CC1+ oligodendrocyte differentiation in the corpus callosum, mosaic pictures of four sections encompassing the corpus callosum were captured using Zeiss Axiosview software with a 10× objective. CC1+ hNA+ cells were counted by counting cells in midline and lateral regions in the corpus callosum; more than 1,500 cells per animal were counted. MBP ensheathment of host axons was assessed in the corpus callosum as described previously (3). In brief, a 2-μm stack of 20 optical sections was obtained every 0.1 μm, and the proportion of ensheathed axons that crossed three perpendicular sampling lines placed randomly over each image was counted (Zeiss LSM 510 Meta NLO confocal microscope).

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Supporting Information

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SI Materials and Methods

**Doxycycline-Inducible TF Expression.** The transcription factor SOX2 coding sequence (CDS) was removed from FUW-tetO-lox-hSOX2 plasmid (Addgene; 20729) (1) using EcoR1 digestion. The NKX2.2 CDS was removed from pTRIP-EF1α-NKX2.2 by EcoR1 digestion and inserted into the vector by ligation to generate FUW-tetO-lox-NKX2.2. The presence of flanking NheI/XhoI in the NKX2.2 sequence permitted the cloning of all genes. All remaining human transcription factor (TF) CDSs were removed from the corresponding pTRIP-EF1α plasmid using common NheI/XhoI sites and ligated into the FUW-tetO-lox-NKX2.2 plasmid. Tet-O-FUW-EGFP (Addgene; 30130) (2) served as a negative control. Human primary cells were co-infected with FUW-M2rtTA virus (Addgene; 20342) and individual TF viruses, each at 1 multiplicity of infection (MOI), for 24 h, followed by complete media replacement. Every other day for 4 d, 3.9 μM doxycycline (VWR International; AAJ60579-14) was added to the culture medium to induce transient TF over-expression.

**Infected NPC Expansion as OPCs and Differentiation into Oligodendrocytes.** Primary human NPCs were infected with either SOX10-HA– or mCherry-expressing lentivirus. Infected NPCs were then maintained in PDGF-AA and FGF2 containing serum-free medium for up to three passages in vitro (approximately 1 mo). At each passage, separate cultures were pulsed with 30 μM BrdU (Sigma-Aldrich; B5002) for 24 h before fixation. To determine whether dividing cells retained the capacity for differentiation, parallel cultures that had been pulsed with BrdU were allowed to differentiate for 4 d following growth factor withdrawal. Cultures were live stained for O4, and after fixation, HA (1: 600, mouse IgG3; Millipore) and BrdU (1:1,000, rat IgG; Serotec) immunofluorescence staining was performed as described in Materials and Methods.


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**Fig. S1.** Identification of human OPC-specific TFs. TFs were identified following genomic analysis of human sorted progenitor populations. Comparison of CD140a+OPCs and CD133+CD140a−NPCs as well as among CD140a/O4 defined-OPCs relative to double-negative cells was used to identify TF candidates (>3 fold change, 5% false discovery rate). Gene Ontology annotation was used to identify DNA-binding proteins and TFs. Further filtering was performed following a PubMed-based literature search to remove proteins with no known TF activity and focus on TFs with potential binding sites in OPC-specific enhancers/promoters. We chose TFs whose expression was regulated among multiple comparisons; the top candidates are shown in both datasets using a heatmap. Each column represents mRNA expression from a single fetal human brain FACS preparation. TFs in bold type were used in further functional analyses.
Fig. S2. Quantitative PCR validation of TF expression in human sorted progenitors. Following microarray analysis, the gene expression profiles of selected TFs were confirmed by real-time quantitative RT-PCR. Gene expression was analyzed immediately after multicolor FACS with CD133/CD140a (A and C) and CD140a/O4 (B and D) antigens. (A and B) Known oligodendrocyte progenitor TFs SOX10, NKX2-2, and OLIG2 were very highly expressed by CD140a+ OPC and O4+ oligodendrocyte fractions. (C and D) Expression of putative OPC TFs PRRX1, TCF12, and RFX4 was confirmed in CD140a+ OPCs, whereas POU2F1 mRNA was not enriched. Data are mean ± SEM shown following ΔΔCt normalization to double-negative cells (n = 3-5 fetal tissue samples/sort).

Fig. S3. Transient overexpression of SOX10, but not ASCL1 or PRRX1, induced O4+ oligodendrocytes. Primary human NPCs were infected with doxycycline-inducible FUW lentivirus after seeding, and doxycycline was supplied for 4 d and removed afterward to stop gene expression. Cells were fixed either at day 7 for NG2 immunofluorescence staining (A–E) or following mitogen removal at day 14 for O4 (F–J). The 4-d overexpression of ASCL1, PRRX1, or SOX10 induced NG2 expression compared with FUW-GFP control (A–D); however, transient overexpression of ASCL1 or PRRX1 did not yield functional OPCs capable of oligodendrocyte differentiation following growth factor removal at day 14 (F–H). Constitutive expression of OLIG2 in ASCL1-expressing cells yielded a high NG2% value (E), but did not induce oligodendrocyte fate (J). In contrast, temporary overexpression of SOX10 was sufficient to induce oligodendrocytes (I). (Scale bar: 50 μm.)
SOX10 overexpression induced proliferating OPCs. Primary human NPCs were infected with SOX10-HA–expressing and mCherry-expressing lentivirus. Infected NPCs were maintained in PDGF-AA– and FGF2-containing medium and allowed to expand for three passages in vitro (>1 mo). Differentiation was stimulated after each passage by removing growth factors for 4 d. To track proliferating progenitor cells and their differentiating progeny, distinct cultures were BrdU-pulsed for 24 h in growth factor and fixed immediately thereafter (A–D and G–J) or following differentiation (E, F, K, and L), for a 4-d chase period. In each condition, OPCs and differentiated oligodendrocytes were stained with NG2 (green, A and G) and O4 (green, C–F and I–L), respectively. Viral SOX10-HA expression was accessed by HA immunofluorescence (red) and BrdU (white). Importantly, although SOX10-HA induced OPC marker NG2, the induced OPCs could be maintained as dividing progenitors (triple-positive cells labeled with arrowheads) for up to three passages in the presence of PDGF-AA and FGF2, but did not differentiate. At each passage, these dividing cells underwent rapid oligodendrocyte differentiation only after removal of mitogens (arrowheads).

(Scale bar: 25 μm.)

MBP protein localization was regulated by SOX10 expression in human NPCs. Human oligodendrocytes that differentiated from NPCs infected with mCherry or SOX10 lentivirus were identified using MBP antibody. Confocal microscopy was used to visualize the intracellular localization of MBP in individual cells. (A) MBP was localized to both the myelin sheath and cell bodies of oligodendrocytes from mCherry-infected cells. (B) In contrast, no cell body MBP staining was seen in oligodendrocytes derived from SOX10-infected donor cells. (Scale bar: 20 μm.)
Fig. 56. SOX10 increases the rate of OPC specification and oligodendrocyte differentiation from human NPCs at 8 wk. mCherry or SOX10 lentivirus-infected human NPCs were injected into neonatal shiverer/rag2 mice and examined at 8 wk. (A–D) Donor-derived OPCs were detected by human specific NG2 staining (green) in both corpus callosum (CC; A and B) and fimbria (C and D). Many more NG2⁺ cells were observed in SOX10 mice (B and D) compared with mCherry control mice (A and C). (E and F) Differentiated human oligodendrocytes were double-stained with hNA (red) and CC1 (green); representative human oligodendrocytes are highlighted with arrows in D. (G) Percentage of hNA⁺CC1⁺ cells among hNA⁺ cells (mean ± SEM; n = 4–6). *P < 0.05 vs. mCherry control, unpaired two-tailed t test. (H–K) Several MBP⁺ mature oligodendrocytes (green) were found in SOX10 mice compared with very few in mCherry mice in corpus callosum (H and I) and fimbria (J and K). (Scale bars: 100 μm in A, B, and E–K; 50 μm in C and D.)
Fig. S7. SOX10 overexpression in primary human OPCs induces rapid oligodendrocyte differentiation. Human CD140a-sorted OPCs immediately after isolation were cultured in serum-free medium for 24 h and then infected with either mCherry- or SOX10-expressing lentivirus (1 MOI; n = 4 fetal brain preparations). At 4 d postinfection, cultures were stained for O4 (green) and GFAP (red) to determine oligodendrocyte differentiation (A and B) and astrocyte differentiation (D and E), respectively. The proportion of human OPCs undergoing differentiation was counted (C and F). SOX10 directly induced oligodendrocyte differentiation among human OPCs and concurrently blocked astrocytic commitment. *P < 0.05 vs. mCherry control, paired t test. (Scale bars: 100 μm; 20 μm in Inset.)

Table S1. Summary of the effects of each TF overexpressed in human NPCs

<table>
<thead>
<tr>
<th>TF</th>
<th>SOX10-MCS enhancer (Fig. 1)</th>
<th>OPC markers at 7 d (Fig. 2)</th>
<th>OPC genomic signature (Fig. 3)</th>
<th>OL differentiation following mitogen withdrawal (Fig. 4)</th>
<th>In vivo proliferation (Ki67/PCNA) (Fig. 6 and Fig S6)</th>
<th>In vivo OPC and OLs (Fig. 5 and Figs. S5 and S6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASCL1</td>
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<td>↑↑</td>
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<td>NKX2.2</td>
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<td>OLIG2</td>
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<td>PRRX1</td>
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<td>SOX10</td>
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<td>TCF12</td>
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</table>

↑↑↑↑ and ↑↑↑↑ represent significant induction; ↑↑, small nonsignificant induction; ~, no change; ↓, small decrease relative to NPCs. Blank cells indicate that the effect of factor overexpression was not determined. OL, oligodendrocyte.
### Table S2. qPCR primers and Taqman assays used in this study

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence (5′ to 3′)</th>
<th>Taqman assay</th>
</tr>
</thead>
</table>
| GAPDH  | Forward: GTGAAGGTGCGAGTCAACGG  
Reverse: CTCGGACCTCAGAACCAGTTGGTG |
| PDGFRA | Forward: CTCGAGCTTCTGGATGATGT  
Reverse: ATACCTCGGTTTCTGTATTTCAAAAT |
| PROM1  | Forward: CAGACTACAAGGCCAAGAACC  
Reverse: AAATCAGATGAGGCCAGCAG |
| CSG4   | Forward: CTTCACTCAGCAGAGTGCTAC  
Reverse: GAGGACAGCTGAGCTCTAGGT |
| NKX2-2 | Forward: GACAATGCTGCGAGATTTGG  
Reverse: AGCCCAAAAGAAGAGAGTGGGAG |
| OLIG2  | Hs00300164_s1 |
| POU2F1 | Hs00231250_m1 |
| RFX4   | Forward: ACCTCACCAGGGAGAGGTGTAG  
Reverse: CCTGGCGAATGCTGAGCTCTT |
| SOX8   | Hs00232723_m1 |
| SOX10  | Hs00366918_m1 |
| TCF12  | Forward: GGCAAGAGCTTCTGGATG  
Reverse: CTGGTTGCGGTCTAGGAAGA |

### Table S3. Summary of lentiviral TF cloning and validation: source and PCR primers used for cDNA amplification and cloning into pTripEF1α-mCherry lentiviral plasmid

<table>
<thead>
<tr>
<th>Gene</th>
<th>cDNA</th>
<th>REs</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Method</th>
</tr>
</thead>
</table>
| ASCL1| Human WM | Nhe1, Xho1 | GCTAGCCATGAAAGCTCTGCAGAA  
Reverse: CTCGGACCTCAGAACCAGTTGGTG | RT-PCR |
| NKX2.2 | BC075092 | Nhe1, Xho1 | AAAGCTAGCCATGATGGAACCAACACAAAGACG  
Reverse: AAATCAGATGAGGCCAGCAG | qPCR, ICC |
| OLIG2 | BC036245 | Nhe1, Xho1 | AAAGCTAGCCATGATGGTGACCCAGCAGGCAAC  
Reverse: AAATCAGATGAGGCCAGCAG | ICC |
| POU2F1 | BC001664 | Xba1, Xho1 | AAAGCTAGCCATGAAAGCTCTGCAGAA  
Reverse: AAATCAGATGAGGCCAGCAG | qPCR |
| RFX4 | Hs00300164_s1 |
| SOX8 | Hs00231250_m1 |
| SOX10 | Hs00366918_m1 |
| TCF12 | Human WM | Spe1, Xho1 | AAAGCTAGCCATGAAAGCTCTGCAGAA  
Reverse: AAATCAGATGAGGCCAGCAG | qPCR |
| RXF4 | Human WM | Nhe1, Xho1 | AAAGCTAGCCATGAAAGCTCTGCAGAA  
Reverse: AAATCAGATGAGGCCAGCAG | qPCR |

TF coding sequences were PCR-amplified from plasmids purchased from Open Biosystems or from human WM extracted reverse-transcribed cDNA. PCR products contained unique restriction enzymes for subsequent cloning into lentiviral plasmid backbone. Following viral production, the expression of each factor was confirmed in HEK 293 cells using a variety of techniques. ICC, immunocytochemistry; RE, restriction enzyme; WM, white matter.