Transcription factor–mediated reprogramming of fibroblasts to expandable, myelinogenic oligodendrocyte progenitor cells

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Cell-based therapies for myelin disorders, such as multiple sclerosis and leukodystrophies, require technologies to generate functional oligodendrocyte progenitor cells. Here we describe direct conversion of mouse embryonic and lung fibroblasts to induced oligodendrocyte progenitor cells (iOPCs) using sets of either eight or three defined transcription factors. iOPCs exhibit a bipolar morphology and global gene expression profile consistent with bona fide OPCs. They can be expanded in vitro for at least five passages while retaining the ability to differentiate into multiprocessed oligodendrocytes. When transplanted to hypomyelinated mice, iOPCs are capable of ensheathing host axons and generating compact myelin. Lineage conversion of somatic cells to expandable iOPCs provides a strategy to study the molecular control of oligodendrocyte lineage identity and may facilitate neurological disease modeling and autologous remyelinating therapies.

Myelin loss or dysfunction affects millions of people worldwide and causes substantial morbidity and mortality. Diseases of myelin in the central nervous system (CNS) are often severely disabling and include adult disorders such as multiple sclerosis and childhood diseases such as cerebral palsy and congenital leukodystrophies. Oligodendrocyte progenitor cells (OPCs), the predominant source of myelinating oligodendrocytes in the CNS, have shown promise as a cellular therapeutic in animal models of myelin diseases1–3. However, sources of OPCs have been restricted largely to allogeneic fetal cells with limited expansion capacity4. Thus, technologies to generate scalable and autologous sources of OPCs are of great interest as they would enable large-scale drug screening and cell-based regenerative medicine. Methods based on pluripotent stem cells or direct lineage reprogramming may meet these requirements.

Recently, we showed efficient differentiation of mouse pluripotent stem cells into pure populations of expandable, myelinogenic OPCs using defined developmental signals5. In the present study, we sought to apply our understanding of oligodendrocyte development to directly convert mouse fibroblasts to expandable OPCs by forced expression of a small number of transcription factors (TFs) (Fig. 1a). Several studies have laid the foundation for the use of lineage conversion in regenerative therapies for neurological disorders6–15. Although these reprogramming technologies have been applied to generate various neuronal fates, such as neurons and neural stem cells, production of myelinogenic OPCs has remained elusive. Here we show that defined sets of transcription factors can reprogram mouse fibroblasts into myelinogenic iOPCs. With further optimization, this approach could provide a source of functional OPCs that will complement, and possibly obviate, the use of pluripotent stem cells and fetal cells in cell-based remyelinating therapies.

RESULTS

Expression of oligodendrocyte-lineage TFs in fibroblasts

Using microarray data5,16, we identified TFs highly enriched in each of the three major CNS lineages: astrocytes (29 TFs), neurons (13 TFs), and OPCs and oligodendrocytes (52 TFs) (Fig. 1b and Supplementary Table 1). We selected eight TFs from the OPC and oligodendrocyte lists on the basis of their known roles during oligodendrocyte development or their ability to enhance oligodendrogenesis when expressed in neural progenitors17,18 and cloned the coding region of each gene individually into a doxycycline-inducible lentiviral vector (Olig1, Olig2, Nkx2.2, Nkx6.2, Sox10, ST18, Gm98 (Myrf) and Myt1; collectively referred to as 8TF) (Supplementary Fig. 1a). The 8TF lentiviral pool was used to infect mouse embryonic fibroblasts (MEFs) isolated from mice constitutively expressing the reverse tetracycline-controlled transactivator (rtTA) and a modified Plp1-eGFP transgene, which is expressed specifically in both OPCs and oligodendrocytes19,20. The Plp1-eGFP; R26-M2rtTA MEFs were carefully isolated to be free of all neural tissue, as demonstrated by the lack of expression of neural stem cell, neuronal, astrocytic, OPC and oligodendrocytic markers by immunostaining, qPCR, microarray and flow cytometry (Fig. 1c, Supplementary Fig. 1b and Supplementary Fig. 2a,b).

In all experiments, we monitored both the percentage of infected cells, by immunostaining of the individual TFs, as well as the transgene induction levels, by qPCR (Supplementary Fig. 1b).

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Typically, 30–60% of cells were infected with an individual factor. Therefore, when cells were infected with multiple viruses, only a small proportion of cells received all TFs. In spite of this, infection of MEFs with the 8TF pool followed by doxycycline induction (a population designated ‘8TF-induced’ MEFs below) consistently resulted in a large percentage of cells expressing the OPC- and oligodendrocyte-specific Plp1-eGFP transgene at day 21 when cells were cultured in defined OPC-promoting culture conditions, containing FGF2, PDGF-AA and sonic hedgehog (SHH) supplements (32.4 ± 9.9%; n = 19 independent biological replicates from 3 independent lots of lentivirus) (Fig. 1c). Uninfected (no TFs) and uninduced (without doxycycline) Plp1-eGFP MEFs cultured under identical conditions for the entire 21-d time course did not express the Plp1-eGFP transgene (Fig. 1c).

8TF-induced fibroblasts exhibit properties of OPCs

We examined the 8TF-induced MEFs for cellular or molecular features consistent with those of bona fide OPCs. During development, OPCs first emerge from the ventral ventricular zone of the spinal cord, have a bipolar morphology, proliferate in response to PDGF and FGF, express a defined set of oligodendrocyte lineage genes and are uniquely able to generate myelinating oligodendrocytes required for CNS myelin maintenance and repair16,21–27. After induction of the 8TF-transduced cells, a subpopulation of the cells underwent a marked morphological change within 21 d, from large, flat, spindle-shaped cells (fibroblasts) to small, bipolar cells, termed iOPCs after further characterization (Fig. 2a,b). We assessed whether the 8TF-induced cells could differentiate into oligodendrocytes in response to growth factor removal and addition of thyroid hormone (T3), a
known inducer of oligodendrocyte differentiation, in vitro\textsuperscript{27,28}. Notably, within 3 d some of the 8TF-induced cells differentiated into cells with a multiprocessed morphology typical of oligodendrocytes (Fig. 2c), called induced oligodendrocytes (iOLs). All iOLs expressed myelin basic protein (MBP), an integral protein component of the myelin sheath, and other defining markers of mature oligodendrocytes, including myelin-associated glycoprotein and myelin oligodendrocyte glycoprotein (Fig. 2d–f).

The efficiency of reprogramming fibroblasts to iOPCs and iOLs is difficult to calculate as the cells proliferate during the 21-d induction time course and only 1–2% receive all 8TFs from the initial lentiviral infections. However, we calculated that, at day 21, ~1 in 900 cells in our bulk 8TF-induced MEF cultures were capable of generating multiprocessed MBP\textsuperscript{+} iOLs after culture in oligodendrocyte differentiation conditions for an additional 3 d (Supplementary Fig. 3a). Generation of iOLs was dependent upon 8TF induction, as uninfected (No TFs plus doxycycline) or uninduced (8TFs without doxycycline) cells never gave rise to iOLs under identical differentiation conditions (Supplementary Fig. 3a).

\textbf{8TF-induced fibroblasts globally express OPC genes}

Although the bulk 8TF-induced MEF cultures at day 21 contained only ~0.1–1% fully reprogrammed cells, as evidenced by the efficiency of forming MBP\textsuperscript{+} iOLs, global gene expression analysis of bulk 8TF-induced MEF cultures showed substantial downregulation of the MEF-specific program and large-scale activation of genes specific to the oligodendrocyte lineage (Fig. 2g). As eight of the OPC-specific genes were initially expressed from our inducible lentiviral vectors, we confirmed, using specific qPCR primers, that the endogenous \textit{Olig2} gene, which is required for oligodendrocyte lineage specification, was activated (Supplementary Fig. 3b). We functionally annotated the gene expression changes caused by 8TF induction using the genomic regions enrichment of annotations tool (GREAT)\textsuperscript{29}. GREAT analysis of genes upregulated in 8TF-induced MEF cultures showed significant association (all $P < 1 \times 10^{-5}$) with Gene Ontology (GO) biological processes, such as “myelination” and “gliogenesis”; with Mouse Genome Informatics (MGI) phenotype ontology terms associated with “oligodendrocyte morphology” and “glial cell morphology”; with MGI expression ontology terms, such as “TS22 spinal cord; lateral wall; ventricular layer”; and with Disease Ontology terms, such as “demyelinating disease” and “schizophrenia” (Supplementary Fig. 3c, see Supplementary Table 2 for the full list of GREAT results).

Genes downregulated in 8TF-induced MEF cultures showed significant association with a large number of mesodermal processes, consistent with inactivation of the global fibroblast gene expression program (Supplementary Fig. 3d, and Supplementary Table 2 for the full list of GREAT results).

\textbf{8TF-induced fibroblasts generate compact myelin}

We studied the ability of 8TF-induced MEFs to myelinate axons of hypomyelinated shiverer (\textit{Mbp}\textsuperscript{shm/shm}) mice, which completely lack MBP and compact myelin and serve as a model of congenital dysmyelinating disorders\textsuperscript{30}. Compact myelin is required for effective saltatory conduction of action potentials along nerve fibers. We first transplanted the cells into organotypic slice cultures of early postnatal shiverer forebrain in vitro (Fig. 3a)\textsuperscript{5,31,32}. The cells engrafted into forebrain slices, colonized major white-matter tracts, including the corpus callosum, and generated characteristic aligned MBP\textsuperscript{+} myelin sheaths in 10 d (Fig. 3b). Furthermore, ultrastructural analysis by electron microscopy showed that the cells generated multilayered compact myelin sheaths around hypomyelinated shiverer host axons in slice culture (Fig. 3c–e).

We next tested whether 8TF-induced cells could function to myelinate shiverer axons in vivo without continued doxycycline induction of the transgenes. We transplanted $5 \times 10^4$ 8TF-induced cells into the dorsal region of the spinal cord of early-postnatal (P3-4) shiverer mice ($n = 4$) and analyzed them after 9–14 d (Fig. 3f). Transplanted cells colonized the dorsal column white matter of shiverer mice and appeared to generate compact myelin sheaths around dorsal column axons (Fig. 3g,h). As shiverer mice are devoid of MBP and therefore lack compact myelin in the CNS, definitive proof of myelination by transplanted cells requires more detailed analysis. We therefore stained sections of shiverer spinal cord transplanted with 8TF-induced cells and found that the myelin produced was MBP\textsuperscript{+} (Fig. 3i,j). This shows
that the myelin produced was of donor origin and not derived from shiverer host peripheral Schwann cells that may have migrated into the CNS during transplantation. Moreover, electron microscopy analysis of the produced myelin showed clear evidence of ultrastructurally normal myelin and the presence of major dense lines (Fig. 3k). We further analyzed and quantified the myelin produced by 8TF-induced cells by calculating g-ratios—a g-ratio is the ratio of axon diameter to total diameter of a myelinated fiber. The g-ratios of myelin produced by 8TF-induced cells was indistinguishable from that of wild-type myelin (wild type, 0.69 ± 0.07; shiverer, 0.88 ± 0.05; 8TF in vitro, 0.68 ± 0.07; 8TF in vivo, 0.70 ± 0.07) (Fig. 3l-o).

We noted that the myelin produced from 8TF-induced cells in slice cultures showed clear properties of oligodendrocyte myelin in that individual cells myelinated multiple axons, whereas the same cells transplanted in vivo seemed to myelinate only a single axon, a property consistent with Schwann cell myelination. To explore this issue, we conducted serial block-face scanning electron microscopy on the dorsal column of shiverer mice after transplantation of 8TF-induced cells. Tracing of cell processes and three-dimensional (3D) reconstruction of the resulting images confirmed that individual transplanted cells myelinated only single axons in vivo (Supplementary Movie 1).

Prospective enrichment of expandable iOPCs

We sought to purify the fully reprogrammed iOPCs from the bulk 8TF-induced cultures by immunosorting. As OPGs are typically defined by the expression of cell-surface markers, including PDGFRα (CD140a), NG2 (Cspg4) and A2B5, we attempted to prospectively

Figure 3 Eight TF-induced MEFs function to generate compact myelin. (a) Experimental scheme for testing the ability of 8TF-induced MEFs to myelinate axons in vitro. 8TF-induced MEFs were transplanted into P5 shiverer forebrain slices and cultured for 10 d. (b) Representative immunofluorescent image of MBP+ myelin tracts generated from expanded (passage 3, day 32) 8TF-induced MEFs 10 d after transplantation into coronal forebrain slice cultures of shiverer mutant mice (dorsal spinal cord of shiverer mutant mice. Major dense lines are evident and indicated by black arrowheads. (c–e) Electron micrograph images of multilayered compact myelin (black arrowheads) generated by donor 8TF-induced MEFs 10 d after transplantation into coronal forebrain slice cultures of shiverer mutant mice. (f) Experimental scheme for testing the ability of 8TF-induced MEFs to myelinate axons in vivo. 8TF-induced MEFs were transplanted into the dorsal spinal cord of P3–P4 shiverer mutant mice. (g,h) Representative immunofluorescent image (g) and matched section stained with toluidine blue (tol blue) (h) showing localization of 8TF-induced cells (containing Plp1-eGFP transgene) 9 d after transplantation into the dorsal spinal cord of shiverer mutant mice (n = 4 mice). The pia mater of the dorsal spinal cord is indicated by the dashed lines. (h, inset) Enlarged view of black box from h showing numerous myelinated axons generated from 8TF-induced cells. (i,j) Representative immunostaining of MBP showing that the myelin produced in shiverer hosts 9 d after transplantation with 8TF-induced MEFs is of donor origin (i). Identically processed wild-type (WT) control (j) showing localization of 8TF-induced cells (containing Plp1-eGFP transgene) 9 d after transplantation into the dorsal spinal cord of WT shiverer mice (n = 4 mice). Differences between WT and each of the other groups were compared using a two-tailed Student’s t-test (***P < 2.2 × 10−16 WT versus shiverer. All others not significant). Scale bars, 25 µm (b), 2 µm (c–i, n), 1 µm (d), 500 nm (e), 100 µm (g,h), 10 µm (i, j) and 100 nm (k).
isolate iOPCs using both the Plp1-eGFP transgene and an additional cell-surface marker. Both PDGFRα and NG2 were expressed on uninduced MEFs, but A2B5 was not. We sorted 8TF-induced MEF cultures at day 21 using A2B5 and Plp1-eGFP and found that 2.30 ± 1.62% (8TF: n = 4 biological replicates from two independent lots of lentivirus) of the cells expressing the Plp1-eGFP reporter were A2B5⁺. The A2B5⁺ cells were a near-homogeneous population of bipolar cells with a morphology similar to bona fide OPCs and different from MEFs (Fig. 4a–c). Hierarchical clustering and pair-wise comparisons of global gene expression data showed that 8TF-induced A2B5⁺ cells correlated tightly with bona fide OPCs (Fig. 4d–f). GREAT analysis of gene expression patterns in 8TF-induced A2B5⁺ cells showed significant associations with such patterns in glial-, oligodendrocyte- and myelin-related processes, phenotypes and diseases (Supplementary Table 2).

The 8TF-induced A2B5⁺ cells could be stably expanded in culture for at least five passages. After six passages, 8TF-induced A2B5⁺ cells began to differentiate, senesce and lose Plp1-eGFP expression and were not used further. During passages 1–6, 8TF-induced A2B5⁺ cells could be readily frozen and thawed without any apparent loss of potential. To analyze their myelinogenic potential, we injected 8TF-induced A2B5⁺ cells into organotypic slice cultures of early postnatal shiverer forebrain. Cells that were 8TF-induced and A2B5⁺ preferentially colonized white matter tracts and differentiated into multiprocessed iOLs displaying extensive ensheathment of neuron axons labeled with neurofilament (NF) antibodies within 10 d (Fig. 4g,h).

Reprogramming fibroblasts to iOPCs with three TFs

In an effort to reduce the number of transcription factors required to generate iOPCs, we induced the expression of eight separate 7TF pools, each lacking a single TF from the original 8TF pool (8TFs – 1TF: n = 3 biological replicates), in the Plp1-eGFP/rtTA MEFs and used the percentage of Plp1-eGFP⁺ cells at day 21 as a surrogate assay for reprogramming. Only pools lacking either Sox10 or Olig2 had significant decreases in the percentage of Plp1-eGFP⁺ cells (P < 0.05), indicating that these genes may be required for reprogramming (Fig. 5a and Supplementary Fig. 4a). To determine whether these two factors alone were sufficient for reprogramming, we induced Sox10 and Olig2 individually or in combination. We did not observe Plp1-eGFP⁺ cells at numbers similar to those observed with 8TFs (Fig. 5a and Supplementary Fig. 4b), and the Plp1-eGFP⁺ cells that were produced did not generate any MBP⁺ iOLs when cultured in differentiation conditions.

To investigate whether a third factor in combination with Sox10 and Olig2 would be adequate to produce iOPCs from MEFs, we induced the expression of three-7TF pools. We found that Nkx6.2, when induced with Sox10 and Olig2 (collectively referred to as 3TF), was sufficient to produce Plp1-eGFP⁺ cells (20.8% ± 1.5%; n = 3 biological replicates). After culture in oligodendrocyte differentiation conditions for 3 d, the 3TF-induced cells upregulated the early oligodendrocyte-specific cell-surface marker O4 (9.2% ± 1.5%; n = 3 biological replicates), whereas uninduced and uninfected cells never gave rise to O4⁺ cells under identical culture conditions (Fig. 5b,c). A subset of the 3TF-induced iOPCs also expressed the mature oligodendrocyte marker Olig2.
oligodendrocyte marker MBP under oligodendrocyte differentiation conditions at a rate similar to that of 8TF-induced cells (Fig. 5d,e).

To test whether 3TF-induced iOPCs could differentiate into other CNS cell fates, we cultured the cells in astrocyte- or neuron-promoting conditions in vitro (n = 3 replicates). The cells never gave rise to GFAP+ astrocytes or MAP2+ neurons in these conditions, either in the presence or absence of doxycycline (Fig. 5d). As all 3TF-based experiments were matched to the same viral titer as our 8TF-based experiments, we determined whether increasing the viral titer would enhance reprogramming to iOPCs. A threefold increase in viral titer resulted in a fivefold increase in the percentage of MBP+ cells (Fig. 5e and Supplementary Fig. 5a–c).

![Image](https://example.com/image1.png)

**Figure 5** Sox10, Olig2 and Nkx6.2 are sufficient to reprogram fibroblasts to iOPCs. (a) Summary graph quantifying the percentage of Plp1-eGFP+ cells induced by subsets of the original 8TF pool at day 21 (n = 3 independent biological replicates from one lot of lentivirus). A 3TF pool of Sox10, Olig2 and Nkx6.2 showed the robust ability to induce Plp1-eGFP+ cells from MEFs (20.8 ± 1.5%). Differences between groups were compared using a two-tailed Student’s t-test (***P < 0.02 and *P < 0.05 versus 8TFs +Dox). (b,c) Immunofluorescent (b) and quantification (c) data showing the capacity of 3TF-induced MEFs at day 21 (3TFs +Dox) to respond to differentiation signals (3TFs +Dox differentiated) and generate multiprocessed O4+ oligodendrocytes. Note that the undifferentiated cultures (3TFs +Dox) contain a population of O4+ cells which are largely bipolar. 3TF-uninduced mutant mice. (d) Representative immunofluorescent images showing the differentiation potential of 3TF-induced MEFs (3TFs +Dox) when exposed to three different lineage-inducing conditions. 3TF-induced MEFs differentiated in 3 d into iOLs that expressed MBP when exposed to oligodendrocyte differentiation conditions both in the presence (+Dox) or absence (−Dox) of doxycycline. 3TF-induced MEFs never gave rise to neurons (MAP2) or astrocytes (GFAP) either in the presence (+Dox) or absence (−Dox) of doxycycline when exposed to the respective neuron- or astrocyte-promoting culture conditions. Positive control cell types that were stained simultaneously to ensure function of each antibody: pluripotent stem cell–derived oligodendrocytes (MBP), astrocytes (GFAP) and neurons (MAP2). (e) Quantitative efficiency of 3TF-induced MEFs (3TF +Dox) to differentiate into MBP+ oligodendrocytes when exposed to oligodendrocyte differentiation conditions for 3 d. Data are presented as mean ± s.e.m. of MBP+ iOLs per 4 × 104 cells seeded (n = 10 independent biological replicates from three lots of lentivirus). 3TF +Dox cells generated with a high viral titer showed a parallel increase in the efficiency of generating MBP+ iOLs (3TF +Dox high virus titer; n = 8 independent biological replicates from two lots of lentivirus). (f) Electron micrograph image of multilayered compact myelin generated from day-21, 3TF-induced MEFs 10 d after transplantation into coronal forebrain slice cultures of shiverer mutant mice. (g) Immunofluorescent images of engraftment and morphology of Plp1-eGFP + 3TF-induced MEFs 10 d after transplantation into P5 coronal forebrain slice cultures of shiverer mutant mice. (h) g-ratios were calculated from 3TF-induced MEFs transplanted into shiverer forebrain slices and dorsal spinal cords (0.69 ± 0.07) were compared using a two-tailed Student’s t-test (***P < 2.2 × 10^-16 WT versus shiverer; all others not significant). Scale bars, 25 µm (b,d), 100 µm (g), and 100 nm (f).
We evaluated the reprogramming time-course dynamics and the properties of the 3TF-induced cells. The reprogramming process was largely complete in 10–14 d (Supplementary Fig. 5d). Global gene expression analysis showed that the 3TF-induced cells largely expressed the OPC-specific network of genes, including endogenous Olig2, and downregulated the MEF-specific network in a similar manner to 8TF-induced cells (Fig. 4d and Supplementary Fig. 5c). When transplanted into shiverer forebrain slices, 3TF-induced cells colonized the corpus callosum and differentiated into MBP+ iOLs (Fig. 5f,g and Supplementary Fig. 5f). Notably, the cells generated multilayered compact myelin with proper ultrastructure and g-ratios (0.63 ± 0.09) (Fig. 5f,h).

Finally, as all of our iOPC experiments were first performed on MEFs, we tested the 3TF pool on a different somatic cell type, mouse lung fibroblasts (MLFs) (Supplementary Fig. 6a). 3TF-induced MLFs showed properties consistent with OPCs, were capable of extensive expansion in vitro, and consistently generated MBP+ iOLs at each passage upon growth factor withdrawal and exposure to T3 (Supplementary Fig. 6b,c). Thus, Sox10, Olig2 and Nkx6.2 are sufficient to convert two separate somatic cell types in 14–21 d to iOPCs capable of generating MBP+ myelinogenic oligodendrocytes.

DISCUSSION

Here we show that functional iOPCs can be produced by delivering defined sets of transcription factors to mouse fibroblasts. Specifically, expression of three transcription factors, Sox10, Olig2 and Nkx6.2, is sufficient to convert two different sources of mouse fibroblasts to iOPCs that exhibit morphological and molecular features consistent with that of bona fide OPCs. In contrast to the ability of OPCs to form both oligodendrocytes and astrocytes, the lineage of iOPCs appears restricted to oligodendrocytes as they do not generate neurons or astrocytes when exposed to differentiation conditions in vitro. It remains possible that iOPCs could access neuron or astrocyte fates under other conditions. The myelination capacity of iOPCs was tested using organotypic slice cultures, which provide a complex 3D tissue representative of the CNS. When transplanted to postnatal forebrain slices, iOPCs myelinated multiple host axons and generated compact myelin. In contrast, iOPCs transplanted in vivo into the spinal cord appeared to myelinate only single axons, a property exhibited by Schwann cells in the peripheral nervous system. Although this result was initially surprising, bona fide CNS OPCs are known to produce Schwann-like cells in vivo and myelinate only single axons. Collectively, our data show that iOPCs function in vitro and in vivo to generate compact myelin and that different environments may direct them to myelinate single or multiple axons.

As with most current reprogramming strategies, the efficiency of generating iOPCs is low. We demonstrated that increasing the viral titer of the reprogramming factors resulted in an increase in the efficiency of generating functional iOPCs. This suggests that further refinement of the stoichiometry and expression levels of the reprogramming factors will lead to increased efficiency of functional iOPC production. In spite of the low reprogramming efficiencies, we showed that iOPCs could be prospectively isolated from the bulk reprogramming cultures using the monoclonal A2B5 antibody. Sorted A2B5+ iOPCs could be expanded for up to five passages while maintaining the ability to differentiate into oligodendrocytes and myelinate host axons after transplantation. Immunosorting of expandable iOPCs should facilitate the use of these cells in molecular and transplantation-based studies that require large numbers of cells.

The potential of cell-based therapies for myelin disorders relies on the ability to generate autologous myelinogenic cells for transplantation. The most promising cell source for such therapies is OPCs. Mature oligodendrocytes largely fail to remyelinate host axons after transplantation. Although neural stem cells and induced neural stem cells can generate oligodendrocytes, the efficiency of this process is quite low, and the cells have a propensity to form neurons and astrocytes. In contrast, iOPCs appear restricted to the oligodendrocyte lineage. We have shown that iOPCs integrate into the CNS and myelinate axons of congenitally dysmyelinated mice in vivo after transplantation. However, for iOPCs to have clinical relevance, future studies must extend this reprogramming strategy to human somatic cells and demonstrate extensive CNS myelination and long-term functional benefit to transplant recipients.

METHODS

Methods and any associated references are available in the online version of the paper.

Accession code. GEO: GSE45440

Note: Supplementary information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

F.J.N., A.M.L. and P.J.T. designed the reprogramming strategy and generated all iOPCs; F.J.N., A.M.L., R.T.K. and P.J.T. performed in vitro differentiation experiments; A.V.C., A.Z., F.J.N., A.M.L. and T.M. performed in vivo myelination experiments; A.Z., F.J.N., A.M.L., R.H.M. and P.J.T. performed slice culture myelination experiments; K.W., A.Z. and R.H.M. produced electron microscope images; F.J.N., A.M.L., D.C.F. and P.J.T. generated and analyzed gene expression data; F.J.N., A.M.L., R.H.M. and P.J.T. analyzed all of the data and wrote the paper. All authors edited and approved the final manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper.

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1. Goldman, S.A., Nedergaard, M. & Windrem, M.S. Glial progenitor cell-based treatment and modeling of neurological disease. Science 336, 491–495 (2012).
2. Franklin, R.J. & Ffrench-Constant, C. Remyelination in the CNS: from biology to therapy. Nat. Rev. Neurosci. 9, 839–855 (2008).
3. Windrem, M.S. et al. Neonatal chimerization with human glial progenitor cells can both remyelinate and rescue the otherwise lethal hypomyelinated shiverer mouse. Cell Stem Cell 2, 553–565 (2008).
4. Sim, F. et al. CD140a identifies a population of highly myelinogenic, migration-competent, and efficiently engrafting human oligodendrocyte progenitor cells. Nat. Biotechnol. (2011).
5. Najmi, F.J. et al. Rapid and robust generation of functional oligodendrocyte progenitor cells from epiblast stem cells. Nat. Methods 8, 957–962 (2011).
6. Vierbuchen, T. et al. Direct conversion of fibroblasts to functional neurons by defined factors. Nature 463, 1035–1041 (2010).
7. Pang, Z.P. et al. Induction of human neuronal cells by defined transcription factors. Nature 476, 220–223 (2011).
8. Caiazzo, M. et al. Direct generation of functional dopaminergic neurons from mouse and human fibroblasts. Nature 476, 224–227 (2011).
9. Pfisterer, U. et al. Direct conversion of human fibroblasts to dopaminergic neurons. Proc. Natl. Acad. Sci. USA 108, 10343–10348 (2011).
10. Yoo, A.S. et al. MicroRNA-mediated conversion of human fibroblasts to neurons. Nature 476, 228–231 (2011).

11. Kim, J. et al. Direct reprogramming of mouse fibroblasts to neural progenitors. Proc. Natl. Acad. Sci. USA 108, 7838–7843 (2011).

12. Son, E.Y. et al. Conversion of mouse and human fibroblasts into functional spinal motor neurons. Cell Stem Cell 9, 205–218 (2011).

13. Han, D.W. et al. Direct reprogramming of fibroblasts into neural stem cells by defined factors. Cell Stem Cell 10, 465–472 (2012).

14. Thier, M. et al. Direct conversion of fibroblasts into stably expandable neural stem cells. Cell Stem Cell 10, 473–479 (2012).

15. Lujan, E., Chanda, S., Ahlenius, H., Sudhof, T.C. & Wernig, M. Direct conversion of mouse fibroblasts to self-renewing, tripotent neural precursor cells. Proc. Natl. Acad. Sci. USA 109, 2527–2532 (2012).

16. Cahoy, J.D. et al. A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. J. Neurosci. 28, 264–278 (2008).

17. Liu, Z. et al. Induction of oligodendrocyte differentiation by Olig2 and Sox10: evidence for reciprocal interactions and dosage-dependent mechanisms. Dev. Biol. 302, 683–693 (2007).

18. Zhang, X. et al. Induction of oligodendrocytes from adult human olfactory epithelial-derived progenitors by transcription factors. Stem Cells 23, 442–453 (2005).

19. Mallon, B.S., Shick, H.E., Kidd, G.J. & Macklin, W.B. Proteolipid promoter activity distinguishes two populations of NG2-positive cells throughout neonatal cortical development. J. Neurosci. 22, 876–885 (2002).

20. Beard, C., Hochedlinger, K., Plath, K., Wutz, A. & Jaenisch, R. Efficient method to generate single-copy transgenic mice by site-specific integration in embryonic stem cells. Genesis 44, 23–28 (2006).

21. Bogler, O., Wren, D., Barnett, S.C., Land, H. & Noble, M. Cooperation between two growth factors promotes extended self-renewal and inhibition of oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells. Proc. Natl. Acad. Sci. USA 87, 6368–6372 (1990).

22. Noble, M., Murray, K., Stroobant, P., Waterfield, M.D. & Riddle, P. Platelet-derived growth factor promotes division and motility and inhibits premature differentiation of the oligodendrocyte-type-2 astrocyte progenitor cell. Nature 333, 560–562 (1988).

23. Richardson, W.D., Pringle, N., Mosley, M.J., Westerman, B. & Dubois-Dalcq, M. A role for platelet-derived growth factor in normal gliogenesis in the central nervous system. Cell 53, 309–319 (1988).

24. Noll, E. & Miller, R.H. Oligodendrocyte precursors originate at the ventral ventricular zone dorsal to the ventral midline region in the embryonic rat spinal cord. Development 118, 563–573 (1993).

25. Rowitch, D.H. & Kriegstein, A.R. Developmental genetics of vertebrate glial-cell specification. Nature 468, 214–222 (2010).

26. Watkins, T.A., Emery, B., Mulinyawe, S. & Barres, B.A. Distinct stages of myelination regulated by gamma-secretase and astrocytes in a rapidly myelinating CNS coculture system. Neuron 60, 555–569 (2008).

27. Crang, A.J., Gilson, J. & Blakemore, W.F. The demonstration by transplantation of the very restricted remyelinating potential of post-mitotic oligodendrocytes. J. Neurocytol. 27, 541–553 (1998).

28. Barres, B.A., Lazar, M.A. & Raff, M.C. A novel role for thyroid hormone, glucocorticoids and retinoic acid in timing oligodendrocyte development. Development 120, 1097–1108 (1994).

29. McLean, C.Y. et al. GREAT improves functional interpretation of cis-regulatory regions. Nat. Biotechnol. 28, 495–501 (2010).

30. Chernoff, G.F. Shiverer: an autosomal recessive mutant mouse with myelin deficiency. J. Hered. 72, 128 (1981).

31. Gahwiler, B.H., Capogna, M., Debanne, D., McKinney, R.A. & Thompson, S.M. Organotypic slice cultures: a technique has come of age. Trends Neurosci. 20, 471–477 (1997).

32. Bai, L. et al. Hepatocyte growth factor mediates mesenchymal stem cell-induced recovery in multiple sclerosis models. Nat. Neurosci. 15, 862–870 (2012).

33. Zawadzka, M. et al. CNS-resident glial progenitor/stem cells produce Schwann cells as well as oligodendrocytes during repair of CNS demyelination. Cell Stem Cell 6, 578–590 (2010).
ONLINE METHODS

Isolation of Plp1-eGFP, R26-M2rtTA fibroblasts. Both MEFs and MLFs were isolated at embryonic day 13.5 (E13.5) from embryos generated through timed natural matings between Plp1-eGFP mice and rtTA mice (B6.Cg-Gt(Rosa)26SorTm1(129SceI)Hze/J, Jackson Laboratory). For MEFs, the head, spinal cord and all internal organs were carefully removed to eliminate contamination with any neural precursors. The remainder of the tissue was cut into small pieces and dissociated using 0.125% Trypsin-EDTA (Invitrogen). Cells were expanded for one passage and cryopreserved for future use. MFLs were isolated by dissociating pooled lung lobes using 0.125% Trypsin-EDTA, expanded for two passages and cryopreserved for future use. Both MEFs and MLFs were derived in DMEM supplemented with 10% FBS, 2 mM Glutamax, 1× nonessential amino acids and 0.1 mM 2-mercaptoethanol.

Selection of 8TFs. The following publically available GEO data sets were used for Fig. 1b: GSM241931, GSM241936, GSM241929, GSM241937, GSM241934 and GSM241933 (ref. 16). Putative transcription factors were filtered by selecting genes with both a ‘GO cellular component term’ ‘nucleus’ and a ‘GO molecular function term’ ‘DNA binding.’ Translation factors that were enriched over threefold in a particular lineage were selected and cross-referenced with our own microarray data of stem cell–derived OPCs and oligodendrocytes (GEO data set: GSE1562). Data were then z-scored and plotted in R using the heatmap.2 function of the gplots package.

Production of lentivirus. The mouse coding regions of Myrf, Myt1, Olig1, ST8, Nkx6.2, Olig2 and Sox10 were cloned into the pLVX-Tight-Puro vector (Clontech). VSV-G pseudotyped lentivirus was generated according to the manufacturer’s protocol using the Lent-i-X HT Packaging Mix and Lentiviral-Phos or Cal-Phos Mammalian Transfection Kit (all from Clontech). 293T cells (Clontech) cultured on rat tail collagen I coated plasticware (BD Biosciences) were seeded to a density of 6.0–8.5 × 106 cells/cm² and transfected 16 h later. Individual supernatants containing virus were harvested at 48 and 72 h post-transfection and filtered with 0.45 µm PVDF membrane (Millipore).

iOPC generation. MEFs or MLFs were seeded at 1.3 × 10⁴ cells/cm², allowed to attach overnight and infected with fresh lentivirus supplemented with polybrene (8 µg/ml) four times over a 2-d period. For 8TF infection, an equal volume of fresh lentiviral supernatant was mixed from each of the 8TFs before infection. To facilitate the comparison of data between 3TF and 8TF experiments, 3TF infections were done by mixing equal volumes of fresh lentiviral supernatant with 8 µl/ml polybrene (8 TFs) before infection. To attach overnight and infected with fresh lentivirus supplemented with polybrene (8 µg/ml) four times over a 2-d period. For 8TF infection, an equal volume of fresh lentiviral supernatant was mixed from each of the 8TFs before infection. To facilitate the comparison of data between 3TF and 8TF experiments, 3TF infections were done by mixing equal volumes of fresh lentiviral supernatant with 8 µl/ml polybrene (8 TFs) before infection.

Immunocytochemistry. Cells were prepared for immunostaining by fixation in 4% paraformaldehyde (Electron Microscopy Sciences) for 15 min and subsequent permeabilization for 10 min with 0.2% Triton-X in PBS. Cells were then blocked for nonspecific binding with filtered 10% normal goat (Abcam) or 10% donkey serum (Abcam) in PBS for 1–2 h at room temperature. Primary antibodies were diluted in blocking solution and incubated with the samples overnight at 4 °C. Samples were rinsed with PBS and incubated with the appropriate fluorescently labeled Alexa-Fluor secondary antibodies (Invitrogen, 1:500) for 1 h at room temperature. Nuclear staining samples were incubated with 1 µg/ml DAPI (Sigma) for 5 min. Primary antibodies used were: Sox10 (R&D Systems, AF2864; 2 µg/ml), Olig2 (Millipore, AB9610; 1:1,000), Nkx6.2 (Abcam, ab58708; 1 µg/ml), Sox1 (R&D Systems, AF3369; 1 µg/ml), Sox2 (R&D Systems, MBA2018; 1 µg/ml), Pax6 (Covance, PRB-278P; 0.67 µg/ml), Oct3/4 (Santa Cruz, SC-5279; 0.4 µg/ml), Nkx2.2 (DSHB, 74.5A5; 4.4 µg/ml), GFAP (DAKO, Z0334; 0.58 µg/ml), MAP2 (Millipore, AB5622; 2 µg/ml), MBP (Covance, SMI-99P; 2 µg/ml), BMP (Abcam, ab7349; 1:100), myelin-associated glycoprotein (Millipore, AB1567; 10 µg/ml) and myelin oligodendrocyte glycoprotein (Millipore, AB5680; 5 µg/ml). For O4 staining, cells were incubated live with 10% donkey serum and O4 antibody (Miller Lab; 1:10) for 20 min. Cells were then gently rinsed three times with cell medium and fixed with 4% PFA in PBS. Staining was then completed as described above.

FACS and flow cytometry. For Plp1-eGFP expression analysis, cells were analyzed on a FACSaria or LSR flow cytometer (BD Biosciences) and plots were generated with WinList 3D 7.0 software. Gates for Plp1-eGFP were set with negative control cells (wild-type MEFs without a GFP transgene) at <0.1% positive cells. For A2B5 immuno staining, cells were collected from culture and blocked in 10% normal donkey serum for 30 min. Cells were then stained with A2B5 primary antibody (R&D Systems, MBA146; 5 µg/ml) for 30 min followed by incubation with Alexa Fluor–labeled secondary antibody (Invitrogen; 4 µg/ml) for 20 min. Isotype control antibody was used as a staining control and to set gates (Mouse IgM, Invitrogen; 5 µg/ml) with Alexa Fluor secondary antibody (Invitrogen; 4 µg/ml). Some experiments were carried out with APC-conjugated A2B5 (Miltenyi Biotec, 130-093-582; 1:11) and conjugated isotype control (Miltenyi Biotec, 130-093-176; 1:11). PDGFRα and NG2 flow cytometry was carried out as detailed previously.

RNA isolation and qPCR. Total RNA was isolated as for gene expression analysis. 400 ng of RNA was reverse transcribed with SuperScript III Reverse Transcriptase (Invitrogen) and qPCR was performed using 8 ng of cDNA with TaqMan Gene Expression Master Mix and TaqMan probes: Sox10 (Mm01300162_m1), Nkx6.2 (Mm00807812_g1), Olig1 (Mm04975371_s1), Nkx2.2 (Mm0127962_m1), Myt1 (Mm00456190_m1), ST8 (Mm01236999_m1), Gm08 (Myr7) (Mm01194559_m1) and Olig2 (AJVI3GC, custom) on the 7300 Real-Time PCR System (Applied Biosystems). Endogenous Olig2 expression was detected using the Olig2 (Mm01120556_m1) TaqMan probe in which one primer sits outside of the CDS and therefore does not detect expression from the Olig2-inducible lentiviral vector. All expression data were normalized to Gapdh (Mm99999915_g1) and samples in which no expression was detected were given an arbitrary Ct value of 40. All analyses were performed with quadruplicate technical replicates for each of a minimum of three independent biological replicates. Relative expression levels were determined by calculating 2−ΔΔCt with corresponding s.e.m.

Global gene expression. Cells were lysed in 1 ml TRIzol (Invitrogen) and stored at -80 °C until ready for use. Chloroform separation was enhanced with Phase-Lock Gel Tubes (5 Prime). The aqueous phase was collected and the RNA isolation completed with the RNeasy Plus Kit (Qiagen) according to...
the manufacturer’s protocol. Sample labeling and hybridization to Affymetrix Mouse Gene 1.0 ST arrays (containing probes covering 28,855 mouse genes) were carried out in the Gene Expression and Genotyping Core Facility of the Case Comprehensive Cancer Center. Data were extracted, and Robust Multi-array Average (RMA) normalized using Affymetrix Expression Console software (ver. 1.1). For heat maps in Figs. 2g and 4d, data were z-scored and plotted in R using the heatmap.2 function of the gplots package. Plots were ranked ordered by the ratio of MEF expression to OPC expression where MEF-specific genes were at the top and OPC-specific genes were at the bottom. Genes were removed from the analysis if signal was not detected above background in any of the six samples analyzed. The plots therefore included values for 13,919 genes. To analyze the global changes during reprogramming, genes increased or decreased twofold between 8TF-induced cells or 8TF A2B5+ iOPCs and MEFs were calculated. Files in BED format containing the transcription start sites ± 100 base pairs of all the genes within each class (genes upregulated in 8TF-induced cells, genes downregulated in 8TF-induced cells, genes upregulated in A2B5+ iOPCs and genes downregulated in A2B5+ iOPCs), were assembled and compared to the background set using the GREAT (http://great.stanford.edu). All significantly associated annotation terms and associated P values are provided in Supplementary Table 2.

Organotypic slice culture myelination analysis. Experiments were carried out as described previously.3 The forebrains of early postnatal day 5 homozygous shiverer (C57Fe/SCW-Mhp/+ (Mhpsh); Jackson Laboratory) were dissected and 300-µm slices produced on a Leica Vibratome. Slices were cultured in a DMEM/BME base supplemented with 15% horse serum, modified N-2 supplement and PDGF-AA for 3 d5,4. 2 × 10^5–2 × 10^6 cells (8TF-induced, 3TF-induced, A2B5+ 8TF-induced or uninduced controls) were manually transplanted with a pulled glass pipette into each slice and grown for an additional 10 d in culture. For staining, slices were then fixed in 4% paraformaldehyde, treated with ice-cold 5% acetic acid/95% methanol and assayed for MBP expression (Covance, SM199 and/or SM194; 4 µg/ml) with either enzymatic secondary antibody (Jackson Labs, Biotin-to-mouse IgG; Vector Labs, ABC, Sigma, DAB) or Alexa-Fluor secondary antibody (Invitrogen; 4 µg/ml). For confocal imaging of IPC ensheathment of axons, d10 A2B5+ iOPC-transplanted forebrain slices were fixed in 4% paraformaldehyde, treated with 0.2% Triton-X and labeled with neurofilament cocktail (Covance, SMI-311 and SMI-312; 1:1,000) and GFP (Aves Labs, 1020; 10 µg/ml) antibodies overnight. Alexa-Fluor secondary antibodies (Invitrogen; 4 µg/ml) were used for detection. Slices were mounted using Vectashield (Vector Labs) and images were acquired with a Zeiss LSM 510 META laser scanning confocal microscope (Carl Zeiss MicroImaging, Jena, Germany) using a 40× O-Achromat, NA 1.2, water immersion objective. All images presented are maximum intensity projections of a Z series consisting of 1-µm optical slices collected every 0.5 µm. Although Triton-X was necessary for the NF and GFP antibodies, it is known to disrupt membrane proteins (that is, myelin) and therefore its use was mimimized as much as possible when staining forebrain slices. For electron microscopy, slices were fixed in electron microscopy fixative (4% paraformaldehyde and 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4) and incubated overnight. Sections were prepared for electron microscopy by incubation in 1% osmium tetroxide and staining en bloc in uranyl acetate and then dehydrated and embedded in Poly/Bed 812 epoxy. Thick (1 µm) transverse sections were cut and stained with toluidine blue. Thin (90 nm) sections were cut, collected on 300-µm nickel grids and carbon-coated for electron microscopy on a JEOL JEM-1200-EX electron microscope. G-ratios were calculated using image analysis software (Adobe Photoshop) by dividing axon diameter with total diameter of the myelinated fiber.

Immunostaining of MBP in epiyembodied sections. 0.5 µm and 1 µm thick sections of Poly/Bed 812 embedded wild-type and cell-injected shiverer spinal cord were etched using a modification of a process described previously.35 Sections were treated with Target Antigen Retrieval solution (DAKO, pH 9.0) and rinsed with 1× D-PBS (Cellgro). They were then incubated in a 1:200 dilution of anti-Mycel Basic Protein antibody (SMI-94, Covance) for 4 nights at 4 °C, rinsed several times with 1× D-PBS (Cellgro) and incubated at ambient temperature in a 1:200 dilution of horseradish peroxidase–conjugated donkey anti-mouse IgG antibody (Jackson ImmunoResearch) for 1.5 h. The staining was visualized using diaminobenzidine (SigmaFast kit). The sample was imaged using transmitted light and oil immersion lens at 100× on a Leica DM 5000B light microscope with a Leica DFC 500 camera.

Serial block face scanning electron microscopy and 3D reconstruction. Sections of cell-injected spinal cord were prepared for 3D electron microscopy using a modified version of previously published methods.36,37 Samples were post-fixed in 2% paraformaldehyde and 2.5% glutaraldehyde with 2 mM calcium chloride in 0.1 M cacodylate buffer, pH 7.4. Samples were then incubated in 2% osmium tetroxide, subsequently stained en bloc in uranyl acetate and then Walton’s lead aspartate. They were dehydrated and embedded in Durcupan ACM resin (EMS). Thick (0.5 µm) transverse sections were cut and stained with a 1% toluidine blue/1% sodium borate solution, and the region of interest was determined and trimmed. Serial blockface scanning electron microscopy was done using a Gatan 3View in–chamber ultramicrotome mounted on a Carl Zeiss Sigma VP scanning electron microscope. Images were obtained in 100 nm increments for an ROI bounded by ~50 µm on each side, resulting in a 3D stack of 570 images (actual ROI dimensions: x = 64 µm, y = 76 µm, z = 57 µm). Images were analyzed using Fiji-Image J (NIH) to align the stack, followed by Amira 5.4.2 for segmentation and 3D reconstruction.

34. Mi, S. et al. Promotion of central nervous system remyelination by induced differentiation of oligodendrocyte precursor cells. Ann. Neurol. 65, 304–315 (2009).
35. Yano, S. et al. An antigen retrieval method using an alkaline solution allows immunoelectron microscopic identification of secretory granules in conventional epoxy-embedded tissue sections. J. Histochem. Cytochem. 51, 199–204 (2003).
36. Denk, W. & Horstmann, H. Serial block-face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure. PLoS Biol. 2, e329 (2004).
37. Mikula, S., Binding, J. & Denk, W. Staining and embedding the whole mouse brain for electron microscopy. Nat. Methods 9, 1198–1201 (2012).

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