Video Article

Lineage-reprogramming of Pericyte-derived Cells of the Adult Human Brain into Induced Neurons

Marisa Karow¹, Christian Schichor², Ruth Beckervordersandforth³, Benedikt Berninger^{1,4}

¹Department of Physiological Genomics, Institute of Physiology, Ludwig Maximilians University Munich

²Tumor Biology Lab, Neurosurgical Clinic, Ludwig-Maximilians University Munich

³Institut für Biochemie, Emil-Fischer-Zentrum, Friedrich-Alexander-Universität Erlangen-Nürnberg

⁴Institute of Physiological Chemistry and Focus Program Translational Neuroscience, Johannes Gutenberg University Mainz

Correspondence to: Marisa Karow at marisa.karow@med.uni-muenchen.de, Benedikt Berninger at berningb@uni-mainz.de

URL: <http://www.jove.com/video/51433>

DOI: [doi:10.3791/51433](https://doi.org/10.3791/51433)

Keywords: Neuroscience, Issue 87, Pericytes, lineage-reprogramming, induced neurons, cerebral cortex

Date Published: 5/12/2014

Citation: Karow, M., Schichor, C., Beckervordersandforth, R., Berninger, B. Lineage-reprogramming of Pericyte-derived Cells of the Adult Human Brain into Induced Neurons. *J. Vis. Exp.* (87), e51433, doi:10.3791/51433 (2014).

Abstract

Direct lineage-reprogramming of non-neuronal cells into induced neurons (iNs) may provide insights into the molecular mechanisms underlying neurogenesis and enable new strategies for *in vitro* modeling or repairing the diseased brain. Identifying brain-resident non-neuronal cell types amenable to direct conversion into iNs might allow for launching such an approach *in situ*, i.e. within the damaged brain tissue. Here we describe a protocol developed in the attempt of identifying cells derived from the adult human brain that fulfill this premise. This protocol involves: (1) the culturing of human cells from the cerebral cortex obtained from adult human brain biopsies; (2) the *in vitro* expansion (approximately requiring 2-4 weeks) and characterization of the culture by immunocytochemistry and flow cytometry; (3) the enrichment by fluorescence-activated cell sorting (FACS) using anti-PDGFR- β and anti-CD146 antibodies; (4) the retrovirus-mediated transduction with the neurogenic transcription factors *sox2* and *ascl1*; (5) and finally the characterization of the resultant pericyte-derived induced neurons (PdiNs) by immunocytochemistry (14 days to 8 weeks following retroviral transduction). At this stage, iNs can be probed for their electrical properties by patch-clamp recording. This protocol provides a highly reproducible procedure for the *in vitro* lineage conversion of brain-resident pericytes into functional human iNs.

Video Link

The video component of this article can be found at <http://www.jove.com/video/51433/>

Introduction

As opposed to reprogramming of somatic cells into induced pluripotent stem cells (iPSCs), which in turn are endowed with a plethora of differentiation potentials, direct reprogramming aims for straight conversion of one specific cell type into another. With respect to their application in the context of disease modeling and potential cell-based therapies, both reprogramming approaches have specific advantages and disadvantages. Reprogramming into iPSCs (i) provides a virtually infinite source of cells; (ii) allows for genetic engineering; (iii) endows with a nearly unlimited differentiation potential. However, major disadvantages of iPSCs entail the risk of tumorigenicity of the undifferentiated cells (teratoma formation *in vivo*) and the need for *ex vivo* cultivation and subsequent transplantation if these cells are to be used for cell-based therapies. Conversely, direct lineage-reprogramming is restricted by the lower yield of the desired cells which correlates directly with the number of the targeted cells of the starting population, but possesses the advantage that lineage-reprogrammed cells appear to exhibit no tumorigenic risk upon transplantation^{1,2}; furthermore, direct reprogramming can even be achieved *in situ*, i.e. within the organ where these cells would be required, thus avoiding the need of transplantation.

With this in mind, our lab has pursued the possibility of lineage-reprogramming brain-resident cells into iNs as a novel approach towards cell-based therapies of neurodegenerative diseases. Brain-resident cells that may be potentially considered as cellular targets for lineage-reprogramming comprise different types of macroglia (astrocytes, NG2 cells and oligodendrocytes), microglia, and microvessel-associated cells (endothelial cells and pericytes). We have extensively studied the *in vitro* reprogramming potential of astroglia of the cerebral cortex of early postnatal mice³⁻⁵. In search of similarly suitable cell sources for direct lineage-reprogramming in the adult human brain, we encountered a cell population that can be successfully reprogrammed into iNs and exhibit hallmarks of pericytes. Here we describe a protocol of how to harvest these cells from adult human brain biopsies, to expand and enrich these cells *in vitro*, and finally successfully reprogram a substantial fraction of these *in vitro* expanded cells (in the range of 25-30%) into iNs. Reprogramming can be achieved by simultaneous retrovirus-mediated co-expression of two transcription factors, *sox2* and *ascl1*. These PdiNs were found to acquire the ability of repetitive action potential firing and to serve as synaptic targets for other neurons indicating their capability of integrating into neural networks. Our protocol provides a straightforward procedure for the isolation and lineage conversion of adult human brain pericytes into iNs.

Protocol

1. Isolation and Culturing of Adult Human Brain Cells

Experiments involving human tissue should be performed in accordance with all relevant governmental and institutional regulations regarding the use of human material for research purposes. The present protocol was developed in accordance with the approval by the ethical committee of the Medical Faculty of the LMU Munich and written informed consent from all patients.

This protocol of preparing cultures of the human adult cerebral cortex has been established using specimen of patients of both sexes suffering from temporal lobe epilepsy or other deep-seated non-traumatic, non-malignant lesions. The tissue obtained from the surgical room comprised exclusively the access channel to the brain lesion and therefore is considered healthy. The age range of the patients was 19-70 years.

1. Prepare growth medium by adding heat-inactivated fetal calf serum (FCS) to DMEM high glucose with GlutaMAX to obtain a final concentration of 20% FCS. Add 5 ml penicillin/streptomycin to a total of 500 ml growth medium. Perform this and all subsequent steps requiring sterile culture conditions in an appropriate laminar flow hood.
2. Keep the adult human brain biopsy obtained from the surgical room in Hanks' balanced salt solution with CaCl_2 and MgCl_2 (HBSS) medium including HEPES (10 mM final concentration) on ice until processing. Start processing as soon as possible.
3. To start the dissociation into single cells, transfer the tissue into a 65 mm petri dish and mince into small pieces by using two sterile single-use scalpels. For enzymatic digestion use 3-6 ml TrypLE in a 15 ml conical tube and incubate for 15-30 min at 37 °C in a water bath.
4. Add 1 volume of prewarmed growth medium to facilitate dissociation and gently triturate the solution containing tissue pieces up and down by first using a 5 ml disposable pipette, followed by using a glass Pasteur pipette until homogenization of the cell suspension. Typically, some residual tissue pieces, mostly consisting of white matter, will remain in the suspension.
5. Spin down at 157 x g for 5 min and resuspend the pellet in the appropriate amount of growth medium (10 ml per uncoated T75 culture flask). Use one T75 culture flask for a biopsy of 5-10 mm diameter in size and extrapolate from this in case of larger specimens.
6. Place cells into a 37 °C incubator with 5% CO_2 and 5% O_2 .
7. Replace half of the medium every 3-4 days until cells have reached confluency. This usually takes up to two weeks (referred to as passage 0 [P0]).

2. *In vitro* Expansion and Characterization of Adult Human Brain Cells

1. *In vitro* expansion:
 1. Aspirate the culture medium and wash with phosphate-buffered saline (PBS), kept at room temperature, before adding 3 ml TrypLE.
 2. Incubate at 37 °C for 5-10 min until most of the cells detached. Gently tap the flasks to facilitate lifting of the cells; following addition of 3 volumes of growth medium, transfer the cells into a 15 ml conical tube and spin down at 157 x g for 5 min and resuspend the cells in the appropriate amount of growth medium.
 3. Passage the cells at a ratio of 1:3 for optimal yield into new T75 cell culture flasks.

Note: We have passaged these cells until P5 without any signs of reduced reprogramming efficiency. While at P0 the culture contains a considerable fraction of endothelial cells (as assessed by CD34 expression), at subsequent passages their number becomes negligible.

2. Characterization of adult human brain cultures:
 1. Immunocytochemistry:
 1. For characterization of the cells, seed ~60,000 cells onto poly-D-lysine-coated glass cover slips in 500 µl fresh growth medium in 24-well tissue culture plates and incubate at 37 °C with 5% CO_2 and 5% O_2 .
 2. For immunocytochemical analysis of the cultured human brain cells remove the medium and wash 3x with 1 ml of PBS.
 3. Fix cells by adding 500 µl of 4% paraformaldehyde in PBS for 15 min at room temperature.
 4. Transfer the glass cover slips into an appropriate humidified staining chamber and block with 80 µl PBS containing 3% bovine serum albumin (BSA) and 0.5% Triton X-100 for 1 hr at room temperature.
 5. Add primary antibodies diluted in the same solution (for dilution factors see table of specific reagents and equipment) and incubate overnight at 4 °C or 1 hr at room temperature.

Note: A substantial but varying fraction of the cells at this stage of culturing are immunoreactive for platelet-derived growth factor receptor β (PDGFR β), cluster of differentiation 146 (CD146), NG2 chondroitin sulfate proteoglycan, and smooth muscle actin (SMA), i.e. markers characteristic of microvessel-associated pericytes. Only a minority (<1%) express the astroglial markers glial fibrillary acidic protein (GFAP) and S100 β . Furthermore, at this stage the culture should be devoid of any cells expressing the neuronal marker β III-tubulin. Use multiple primary antibody combinations for co-labeling of different markers. Further confirmation of the expression of glial, neuronal, endothelial and pericyte markers can be obtained by reverse transcription and quantitative real time polymerase chain reaction (not described in this protocol).

6. Following incubation with the primary antibodies wash three times with 1 ml PBS and add the corresponding secondary antibodies (in the appropriate dilutions) to the staining solution and incubate 1 hr at room temperature in the dark. Wash the cells 3x with PBS. If required, prior to mounting include staining with DAPI for 5 min at room temperature.
 7. Use antifading mounting medium and analyze the cover slips using an epifluorescence microscope or a confocal microscope.
2. Flow cytometry:
 1. For analysis of surface marker expression using flow cytometry, grow cells to confluency in uncoated T25 or T75 cell culture flasks.

2. Detach cells by using TrypLE as described before and resuspend in staining solution consisting of PBS + 0.5% BSA. Per staining reaction, resuspend 100,000-200,000 cells in 100 μ l of staining solution. Prepare single staining reactions per antibody used (CD146-FITC, CD140b-PE, CD34-APC, CD13-FITC, respective isotype control antibodies).
3. Add fluorochrome-conjugated antibodies to each staining solution (for dilutions see table of specific reagents and equipment) directly into the cell suspension and incubate at 4 °C for 30 min in the dark.
4. Wash the cells three times with 500 μ l PBS and spin down between each washing step at 157 x g for 5 min.
5. Resuspend the cell pellet in 0.5-1 ml staining solution and transfer cells through a cell strainer (70 μ m) to FACS tubes. Protect the sample from exposure to light.
6. Vortex samples prior to placing them in the FACS instrument.
7. To analyze the living cells and exclude debris and cell aggregates, gate for FSC-A and SSC-A. Cell duplets are specifically gated out by FSC-A and FSC-W. To determine the right gating conditions for the cell surface markers set the gates with isotype-matched antibody control conjugated to the respective fluorochrome. Then determine the proportion of antibody-bound cells.

3. Enrichment for Pericyte-derived Cells by Fluorescence Activated Cell Sorting

1. Purify the pericyte cell population (resuspended in growth medium) prior to transduction via FACS sorting using a 70 μ m nozzle. Use CD34-APC in combination with CD140b-PE and CD146-FITC to select for pericytes. Sort for CD34-negative, CD140b-, and CD146-positive cells.
2. Collect the sorted cells in growth medium and plate onto poly-D-lysine-coated glass cover slips in 24-well tissue culture plates and incubate at 37 °C with 5% CO₂ and 5% O₂.
3. 48 h after sorting replace the medium with fresh growth medium and perform transduction as described in Protocol 4.

4. Retroviral Transduction of Pericyte-derived Cells and Reprogramming into Induced Neurons

Note: Refer to local biosafety guidelines when handling retroviral particles. In Germany the use of the retroviruses employed here require biosafety level 2. For production of retroviral particles refer to protocol⁶. For retroviral constructs used for reprogramming, refer to Karow *et al.* (2012). Retroviruses were pseudotyped with VSV-G (vesicular stomatitis virus-glycoprotein)⁵. For production please refer to Gavrilescu *et al.*⁶.

1. 24 hr prior to retroviral transduction seed ~60,000 cells onto poly-D-lysine-coated glass cover slips in 500 μ l fresh growth medium in 24-well tissue culture plates and incubate at 37 °C with 5% CO₂ and 5% O₂.
2. The next day, replace the growth medium with 500 μ l fresh, prewarmed growth medium and add 1 μ l of the respective concentrated supernatants containing retroviral particles (pCAG-IRES-*dsred* for control, especially during establishing the protocol in the lab), pCAG-*asc1*-IRES-*dsred*, pCAG-*sox2*-IRES-*gfp*.
3. One day later, remove the medium including the viral particles from the cells and add 1 ml fresh, prewarmed B27-differentiation medium, composed of 49 ml DMEM high glucose with GlutaMAX and 1 ml B27 serum-free supplement. Maintain the cells in culture at 37 °C in 5% CO₂ and 5% O₂ for 4-8 weeks without changing the medium as repetitive medium changes become harmful as iNs mature.

5. Characterization of Induced Neurons by Immunocytochemistry

1. To determine the cellular identity of the transduced cells, in particular to demonstrate the acquisition of a neuronal phenotype, perform immunocytochemistry against neuronal antigens such as β -tubulin, MAP2, and NeuN (for antibodies and their respective dilutions, see table of specific reagents and equipment; follow the same procedure as indicated in 2.2.1).
2. For improving the maturation of PdiNs, co-culture these cells with neurons derived from E14 mouse cerebral cortices. To this end, dissect the cerebral cortex and dissociate the tissue mechanically with a fire-polished glass Pasteur pipette. Add 10,000-50,000 murine neurons to cultures of pericyte-derived cells 2-3 weeks following transduction with *sox2*- and *asc1*-encoding retroviruses and continue culturing. Transduced cells can be distinguished from murine neurons by their reporter (GFP and *dsRed*) expression, either by live epifluorescence or following immunocytochemistry for GFP and *dsRed*.
3. To assess the formation of synapses onto PdiNs by murine neurons, perform immunocytochemistry against vesicular neurotransmitter receptors such as vesicular glutamate transporter 1 (vGluT1).
4. Probe transduced cells for their electrical properties (*i.e.* ability of generating action potentials) and the establishment of functional synapses by patch-clamp electrophysiology. For details of the procedure see Heinrich *et al.* (2011).

Representative Results

The first outcome of this protocol after successfully establishing a culture from a specimen of human adult cerebral cortex consists in the identification of the cellular composition of the culture. Immunocytochemistry for cell type specific proteins reveals a considerable degree of heterogeneity between cultures derived from specimen of different patients (**Figure 1A**). As quantified by flow cytometry there is always a substantial fraction of cells that express PDGFR β (on average ~75%, ranging from 30 to up to 99%); other markers of pericytes such as CD146, NG2 and CD13 are typically expressed in a lower range (**Figure 1B**). Likewise SMA is expressed in a minor subpopulation of the cultured cells, as determined by immunocytochemistry (**Figure 1A**). At passage 0 (P0), there are usually <10% of CD34-positive endothelial cells, and their number declines below detection with further passaging. Likewise, astrocytes comprise only a negligible contamination at earlier passages. Furthermore, there are typically no cells that stain for neural stem cell, neuronal, or oligodendrocyte markers. Immunocytochemical and flow cytometry data are fully corroborated by quantitative RT-PCR⁷. In sum, the largest fraction within the culture expresses proteins and mRNAs characteristic of pericytes. A further enrichment for pericyte-derived cells can be achieved by FACS at this stage. This is particularly relevant when the purity of the culture is at the lower end of the spectrum as reflected by the level of PDGFR β expression. Thus, we have utilized an

antibody against PDGFR β (CD140b) alone or in combination with an anti-CD146 antibody, while excluding any CD34-positive contaminants, to FAC-sort specifically pericytes (**Figure 1C**).

Transducing these cultures with control viruses encoding a reporter gene only, does not alter their marker expression profile (assessed 3–4 weeks following transduction), indicating that these cells remain in the pericyte lineage. Likewise, retrovirus-mediated expression of Sox2 alone does not result in any overt changes in morphology nor induces β III-tubulin expression suggesting that Sox2 alone is not sufficient to induce a fate conversion. In contrast, a low percentage (approximately 10%) of the cells transduced with an ascl1-encoding retrovirus alone exhibit β III-tubulin expression, however without acquiring a neuronal morphology. Strikingly, nearly 50% of all sox2- and ascl1-cotransduced cells show β III-tubulin expression and even more importantly, a third of these double-transduced cells (visualized by their double reporter expression) also display neuronal processes (**Figure 2**). Along with the acquisition of neuronal features, expression of PDGFR β is down-regulated⁷. With further maturation in culture, sox2- and ascl1-coexpressing cells also become immunoreactive for the more mature neuronal markers MAP2 (staining dendritic processes) and NeuN (staining predominantly neuronal cell bodies). As discussed below these changes in marker expression also correlate with the acquisition of electrophysiological hallmarks of neurons. In sum, these data provide evidence for the direct fate conversion of pericyte-derived cells into iNs, referred to as PdiNs. These PdiNs possess the competence to establish functional postsynaptic compartments as revealed in coculture experiments with neurons of the embryonic mouse cerebral cortex. This competence can be demonstrated electrophysiologically in the appearance of synaptic inputs (see discussion below) as well as the decoration of dendritic processes of PdiNs with presynaptic terminals derived from co-cultured neurons (characterized by clusters of vesicular neurotransmitter transporters, *e.g.* vGluT1 immunoreactivity). These data further indicate the ability of PdiNs to integrate into neuronal circuits.

Figure 1. Heterogeneous expression of pericyte markers in cultures derived from the adult human cerebral cortex. A) Fluorescent micrographs illustrating the heterogeneous expression of pericyte markers (PDGFR β , SMA, CD146) as revealed by immunocytochemistry. Scale bar = 50 μ m. **B)** The histogram summarizes the relative numbers of cells positive for the pericyte markers PDGFR β (CD140b), CD13, and CD146 as determined by flow cytometry of different patient-derived cultures. CD34 is a marker for endothelial cells. **C)** FACS dot plots depicting isotype control- and CD146/ CD140b-stained human cells for sorting of the double-positive pericytes (highlighted by the red box) for subsequent reprogramming. Note the high rate of PDGFR β expression and the heterogeneity with regard to CD146 expression in this particular culture.

Figure 2. Reprogramming of adult human pericyte-derived cells into iNs. Top: Experimental set-up. Below: Fluorescent micrographs depicting cells transduced with retrovirus encoding *sox2*-IRES-*GFP* and *ascl1*-IRES-*DsRed*. Note that only double transduced cells (arrowheads) express β III-tubulin (right panel) and exhibit neuronal morphology.

	cell division [%]	cell death [%]	β III-tubulin+ [%]
untransduced	36	3	0
Sox2	46	7	0
Ascl1	26	33	7
Sox2-Ascl1	3	36	25

Table 1. Behavior of pericyte-derived cells undergoing conversion into PdiNs as assessed by continuous live imaging. Note that Sox2- and Ascl1 co-expressing cells exit cell cycle. Moreover, note the high rate of cell death following Ascl1 or Sox2 and Ascl1- coexpression. Taking into account these distinct cellular events, approximately 25% of all co-transduced cells become iNs.

Discussion

The present protocol describes the *in vitro* expansion and enrichment of pericyte-derived cells following isolation from the adult human cerebral cortex and the subsequent conversion into iNs by retrovirus-mediated expression of the neurogenic transcription factors Sox2 and Ascl1. Such protocol provides an experimental *in vitro* system to study the lineage-conversion of brain-resident cells into neurons and potentially also glia, with the goal in mind to ultimately translate this direct conversion approach into the *in vivo* setting of the adult brain.

Technical Considerations

In our study we utilized human brain tissue from patients aged between 19 and 70 years, from both sexes. We did not notice a overt difference in reprogramming efficiency depending on gender or age⁷. It is nevertheless recommended to document this information as more subtle differences may nonetheless be observed that escaped our attention.

It is further recommended to commence processing as early as possible following transfer of the tissue sample to the lab. There will be necessarily some unknowns for how long the tissue has remained in the surgical room prior to transfer to the experimentalist. We estimate that in our study⁷ the time between removal from the patient's brain to the start of the culturing procedure ranged between 2-6 hr, with the samples being kept all time on ice. No overt difference in the ease of subsequent processing nor any adverse outcome were noticed within this time window.

A critical assessment for any protocol concerns its simplicity, reproducibility, and efficiency. The present protocol is straightforward in that during a first phase cells are expanded in an inexpensive medium which can be easily obtained, followed by a second phase of cell reprogramming using only two transcription factors and culturing in a defined medium.

Regarding reproducibility, we observed that the quality of the retrovirus preparation is of utmost importance, with low titer virus preparations used at dilutions to match the number of infectious particles of high titer viruses often resulting in failure of reprogramming. In fact we noted that the same pericyte culture in which reprogramming had failed previously could be successfully converted into iNs when using high titer viruses.

Regarding efficiency of conversion, we have assessed the number of β III-tubulin-positive cells derived from sox2- and ascl1-cotransduced cells using continuous live imaging. Live imaging allows for assessing the number of cells that undergo cell division or cell death during the conversion process, while such information is lacking when the number of generated iNs is determined only at the end point of the experiment. In fact using time-lapse video microscopy (for the procedure refer to Ortega *et al.*⁸) it was observed that untransduced and single factor-transduced cells continue proliferating, while sox2- and ascl1-cotransduced cells rapidly exit the cell cycle. Moreover, a significant fraction of the latter population undergoes cell death. While retrovirus toxicity cannot be formally excluded from the data obtained in our study⁷, the fact that increased cell death was observed only when ascl1 was expressed (alone or in combination with sox2), we interpret this as evidence for a catastrophic conflict of cell fates. Taking these two events into account, we estimate that 25% of all sox2- and ascl1-cotransduced cells convert into β III-tubulin positive iNs (Table 1).

As co-transduction is absolutely required for successful reprogramming, one may consider employing a viral vector encoding both genes to optimize the amount of cells expressing both factors at the same time.

Recently, Ladewig *et al.* developed a protocol for the efficient conversion of fibroblasts into iNs obtaining a yield of 200% by using small molecule-based inhibition of glycogen synthase kinase-3 β and SMAD signaling⁹. It will be interesting to determine whether these add-ons to our protocol improve the yield of PdiN production.

Another interesting outcome of the live imaging of the reprogramming process concerns the fact that the conversion of adult human pericytes into iNs occurs at a rather slow tempo compared to similar reprogramming protocols in cultures of different somatic cells of mouse origin⁴. This is consistent with the characteristic slow maturation of human neurons¹⁰.

While we have not systematically analyzed the impact of the oxygen tension on the yield of PdiN production, we noted that incubation in low oxygen conditions (5%) generally gave superior results compared to culturing the cells in normoxic conditions (21%). Recently, Davila *et al.* described a similar enhancing effect by reduced oxygen tension for the induction of iNs from human fibroblasts¹¹.

Our protocol is based on the retroviral delivery of sox2 and ascl1 while other direct conversion protocols have employed inducible lentiviral vectors^{1,12,13}. More recently we have also successfully applied doxycycline-inducible lentiviral constructs encoding sox2 and ascl1, indicating that a relatively short pulse (10 days) of Sox2 and Ascl1 expression is sufficient to cause pericyte-to-neuron conversion. The retroviruses used in our study⁷ were designed for reduced silencing⁵. Accordingly, we did not notice the appearance of neurons devoid of reporter gene expression. However, the possibility that the overall expression levels of both transgenes and reporter change over time cannot be excluded. Integration-free approaches (utilizing Sendai viruses) have been lately employed in case of the generation of induced neural progenitors¹⁴, but it is currently not known whether this or even virus-free reprogramming strategies can be successfully applied to convert human pericytes. Finally, whether pericytes derived from other tissues can undergo conversion into induced neural cell types remains to be tested.

Conceptual Considerations

A critical outcome of reprogramming of somatic cells into iNs concerns their functionality¹⁵. We have assessed the electrophysiological properties of PdiNs at different time points following the onset of the reprogramming process. At very early stages, *i.e.* 2 weeks following retroviral transduction, PdiNs exhibit barely signs of electrical excitability, despite β III-tubulin expression at this stage. Thus, most of the electrophysiological analyses were conducted 4-8 weeks following transduction with sox2 and ascl1. As described in Karow *et al.*, PdiNs are characterized by considerable immaturity as reflected by high input resistances and a low frequency of action potential firing. Further maturation can be promoted by co-culturing PdiNs with neurons derived from the mouse E14 cerebral cortex. Under these conditions, PdiNs exhibit higher action potential frequencies and increased sodium currents⁷. Moreover, coculturing reveals the competence of PdiNs to receive functional synaptic input from mouse neurons. In sum, PdiNs acquire functional neuronal properties which can be further enhanced upon coculturing with other neurons. However, it remains to be shown whether PdiNs can also establish functional presynaptic compartments. Further studies transplanting PdiNs into the brain are needed to reveal their potential to reach full maturity and functionality.

Using sox2 and ascl1 as reprogramming factors we noted that the resulting PdiNs acquire features of GABAergic neurons⁷. Interestingly, other groups have developed protocols to convert fibroblasts into iNs by using different transcription factor and/or microRNA combinations with the result that the developing iNs acquired distinct neurotransmitter specification such as a glutamatergic, dopaminergic, and cholinergic phenotype^{1,16-18}. It remains to be tested whether the factor combinations used in fibroblasts induce the same transmitter identity in PdiNs. Moreover, recent work has expanded the conversion spectrum beyond neurogenesis to generate neural stem cells^{19,20} or glial, in particular oligodendrocytes^{21,22}. If these reprogramming protocols elicit a similar outcome in adult human brain pericyte-derived cells, this would greatly widen the panorama of applications of this important brain-resident cell type.

Disclosures

Authors have nothing to disclose.

Acknowledgements

We are grateful to Dr. Magdalena Götz for her input during the development of this protocol. We thank Dr. Marius Wernig (Stanford University) for generously providing us with the sox2 coding sequence. We are also very grateful to Dr. Alexandra Lepier for virus production. This work was supported by grants of the Deutsche Forschungsgemeinschaft (BE 4182/2-2) and the BMBF (01GN1009A) to B.B., and the Bavarian State Ministry of Sciences, Research and the Arts to M.K. and B.B. C.S. received funding from the binational SYSTHER-INREMOS Virtual Institute (German and Slovenian Federal Ministries of Education and Research) and the DFG (SFB 824).

References

1. Caiazzo, M., *et al.* Direct generation of functional dopaminergic neurons from mouse and human fibroblasts. *Nature*. **476**, 224-227, doi:10.1038/nature10284 (2011).
2. Kim, J., *et al.* Functional integration of dopaminergic neurons directly converted from mouse fibroblasts. *Cell stem cell*. **9**, 413-419, doi:10.1016/j.stem.2011.09.011 (2011).
3. Duan, B., *et al.* Interactions between water deficit, ABA, and provenances in *Picea asperata*. *Journal of experimental botany*. **58**, 3025-3036, doi:10.1093/jxb/erm160 (2007).
4. Heinrich, C., *et al.* Directing astroglia from the cerebral cortex into subtype specific functional neurons. *PLoS Biol*. **8**, e1000373, doi:10.1371/journal.pbio.1000373 (2010).
5. Heinrich, C., *et al.* Generation of subtype-specific neurons from postnatal astroglia of the mouse cerebral cortex. *Nat Protoc*. **6**, 214-228, doi:nprot.2010.188 [pii] 10.1038/nprot.2010.188 (2011).
6. Gavrilescu, L. C., & Van Etten, R. A. Production of replication-defective retrovirus by transient transfection of 293T cells. *J Vis Exp*. (10), e550, doi:10.3791/550 (2007).
7. Karow, M., *et al.* Reprogramming of pericyte-derived cells of the adult human brain into induced neuronal cells. *Cell stem cell*. **11**, 471-476, doi:10.1016/j.stem.2012.07.007 (2012).
8. Ortega, F., *et al.* Using an adherent cell culture of the mouse subependymal zone to study the behavior of adult neural stem cells on a single-cell level. *Nat Protoc*. **6**, 1847-1859, doi:10.1038/nprot.2011.404 nprot.2011.404 [pii] (2011).
9. Ladewig, J., *et al.* Small molecules enable highly efficient neuronal conversion of human fibroblasts. *Nat Methods*. **9**, 575-578, doi:10.1038/nmeth.1972 nmeth.1972 [pii] (2012).
10. Espuny-Camacho, I., *et al.* Pyramidal neurons derived from human pluripotent stem cells integrate efficiently into mouse brain circuits *in vivo*. *Neuron*. **77**, 440-456, doi:10.1016/j.neuron.2012.12.011 S0896-6273(12)01121-X [pii] (2013).
11. Davila, J., Chanda, S., Ang, C. E., Sudhof, T. C., & Wernig, M. Acute reduction in oxygen tension enhances the induction of neurons from human fibroblasts. *J Neurosci Methods*. **216**, 104-109, doi:10.1016/j.jneumeth.2013.03.020 S0165-0270(13)00125-8 [pii] (2013).
12. Vierbuchen, T., *et al.* Direct conversion of fibroblasts to functional neurons by defined factors. *Nature*. **463**, 1035-1041, doi:nature08797 [pii] 10.1038/nature08797 (2010).
13. Pfisterer, U., *et al.* Direct conversion of human fibroblasts to dopaminergic neurons. *Proc Natl Acad Sci USA*. **108**, 10343-10348, doi:1105135108 [pii] 10.1073/pnas.1105135108 (2011).
14. Lu, J., *et al.* Generation of Integration-free and Region-Specific Neural Progenitors from Primate Fibroblasts. *Cell Rep*. **3**, 1580-1591, doi:10.1016/j.celrep.2013.04.004 S2211-1247(13)00171-X [pii] (2013).
15. Yang, N., Ng, Y. H., Pang, Z. P., Sudhof, T. C., & Wernig, M. Induced neuronal cells: how to make and define a neuron. *Cell Stem Cell*. **9**, 517-525, doi:10.1016/j.stem.2011.11.015 S1934-5909(11)00543-1 [pii] (2011).
16. Pang, Z. P., *et al.* Induction of human neuronal cells by defined transcription factors. *Nature*. **476**, 220-223, doi:10.1038/nature10202 nature10202 [pii] (2011).
17. Yoo, A. S., *et al.* MicroRNA-mediated conversion of human fibroblasts to neurons. *Nature*. **476**, 228-231, doi:10.1038/nature10323 nature10323 [pii] (2011).
18. Son, E. Y., *et al.* Conversion of mouse and human fibroblasts into functional spinal motor neurons. *Cell Stem Cell*. **9**, 205-218, doi:10.1016/j.stem.2011.07.014 S1934-5909(11)00377-8 [pii] (2011).
19. 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, doi:10.1073/pnas.1121003109 (2012).
20. Thier, M., *et al.* Direct conversion of fibroblasts into stably expandable neural stem cells. *Cell Stem Cell*. **10**, 473-479, doi:10.1016/j.stem.2012.03.003 (2012).
21. Yang, N., *et al.* Generation of oligodendroglial cells by direct lineage conversion. *Nat Biotechnol*. **31**, 434-439, doi:10.1038/nbt.2564 nbt.2564 [pii] (2013).
22. Najm, F. J., *et al.* Transcription factor-mediated reprogramming of fibroblasts to expandable, myelinogenic oligodendrocyte progenitor cells. *Nat Biotechnol*. **31**, 426-433, doi:10.1038/nbt.2561 nbt.2561 [pii] (2013).