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Fast and Efficient Neural Conversion of Human Hematopoietic Cells

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SUMMARY

Neurons obtained directly from human somatic cells hold great promise for disease modeling and drug screening. Available protocols rely on overexpression of transcription factors using integrative vectors and are often slow, complex, and inefficient. We report a fast and efficient approach for generating induced neural cells (iNCs) directly from human hematopoietic cells using Sendai virus. Upon *SOX2* and *c-MYC* expression, CD133-positive cord blood cells rapidly adopt a neuroepithelial morphology and exhibit high expansion capacity. Under defined neurogenic culture conditions, they express mature neuronal markers and fire spontaneous action potentials that can be modulated with neurotransmitters. *SOX2* and *c-MYC* are also sufficient to convert peripheral blood mononuclear cells into iNCs. However, the conversion process is less efficient and resulting iNCs have limited expansion capacity and electrophysiological activity upon differentiation. Our study demonstrates rapid and efficient generation of iNCs from hematopoietic cells while underscoring the impact of target cells on conversion efficiency.

INTRODUCTION

Cellular reprogramming has opened new avenues to investigate human disease and identify potential targets for drug discovery (Bellin et al., 2012). This technology is particularly useful for cell types in which the target tissue is not accessible, like the brain. It is now possible to differentiate human embryonic stem (hES) and human-induced pluripotent stem (hiPS) cells into different types of neurons (Hu et al., 2010; Qiang et al., 2014; Velasco et al., 2014; Zhang et al., 2013). However, the generation of neuronal cells from pluripotent stem cells involves long and complex protocols with problematic variability. Alternatively, direct lineage conversion (or transdifferentiation) of somatic cells into neurons (induced neurons [iNs]) has been achieved by forced expression of lineage-specific transcription factors and microRNAs (miRNA) (Ambasudhan et al., 2011; Caiazzo et al., 2011; Pang et al., 2011; Pfisterer et al., 2011; Vierbuchen et al., 2010; Yoo et al., 2011). Using this approach, several cell types (Giorgetti et al., 2012; Karow et al., 2012; Marro et al., 2011) have been converted into functional neurons in vitro and also in vivo (Guo et al., 2014; Su et al., 2014; Torper et al., 2013). However, for delivery of exogenous reprogramming factors, most available protocols have used integrative viral vectors, and the conversion process was rather inefficient. Only recently, nonintegrative methods based on Sendai virus (SeV) or chemically defined culture conditions have been described for the direct conversion of nonhuman cells into neural progenitor cells (iNPCs) (Cheng et al., 2014; Lu et al., 2013).

Here, we investigated whether a similar nonintegrative strategy is applicable for the conversion of human hematopoietic cells directly into neurons. Importantly, peripheral blood (PB), which is routinely used in medical diagnoses, represents a noninvasive and easily accessible source of cells for reprogramming both healthy donor and disease-specific patient cells. Based on our previous study (Giorgetti et al., 2012), we chose *SOX2* and *c-MYC* SeV vectors to reprogram CD133-positive cord blood (CB) cells and adult PB mononuclear cells (PB-MNCs). We found that the overexpression of *SOX2* and *c-MYC* by SeV accelerated and increased the efficiency of neural conversion of CD133-positive CB cells (CB-iNCs) when compared with retroviral vectors. *SOX2* and *c-MYC* were also sufficient to convert PB-MNCs into neuronal-like cells (PB-iNCs). However, compared with CB-iNCs, the process was less efficient,

and the resulting PB-iNCs showed limited expansion, differentiation capacity, and functional properties. Our results demonstrate the feasibility for rapid and efficient generation of iNCs from CD133-positive CB cells using nonintegrative SeV while underscoring the impact of target cell developmental stage on the reprogramming process for lineage conversion.

RESULTS

Rapid and Efficient Generation of iNCs from CD133-Positive CB Cells

We first tested whether the forced expression of *SOX2* and *c-MYC* by SeV can induce the conversion of CD133-positive CB cells directly into neural cells (iNCs); 50,000 magnetic activated cell sorting-isolated CD133-positive CB cells (purity >95%; data not shown) were infected at a low multiplicity of infection (MOI) (<5 MOI, infection efficiency 80%–85%; data not shown) and cocultured on irradiated rat primary astrocytes in the presence of N2 medium containing bone morphogenetic protein (BMP), transforming growth factor β (TGF-β), and glycogen synthase kinase-3β (GSK-3β) inhibitors (Ladewig et al., 2012) (Figure 1A). Overexpression of *SOX2* and *c-MYC* by SeV rapidly induced the acquisition of neuroepithelial morphology in CD133-positive CB cells (Figure 1Ba–c). After removal of inhibitors (day 10), reprogrammed cells showed a high expansion capacity, acquired an immature neural morphology (day 15; Figure 1Bd), and progressively formed a neural network. By day 30, CB-iNCs displayed a more complex cytoarchitecture with long processes and elaborated branching, preferentially organized into clusters, with persistence of proliferating cores (Figure 1Be).

Immunostaining showed that by day 15 most cells were positive for SOX2, and some of them already expressed beta-III tubulin (TUJ1) (Figure 1Ca). By day 30, the cells were organized in clusters that expressed TUJ1 and were interconnected by microtubule-associated protein 2 (MAP2)-positive fibers (Figure 1Cb–d); there were some PAX6-positive cells within the clusters (indicated by white arrows; Figure 1Cc). Quantitative analysis revealed that after 30 days of neural induction a majority of HUNU cells were positive for TUJ1 (~80%, n[HUNU cells] = 17,330; two independent experiments) (Figures S1A and S1B available online) and around 50% (n[HUNU cells] = 24,362) were positive for MAP2 (Figure S1C). Moreover, fluorescence-activated cell sorting (FACS) analysis revealed that over 25% of cells were neural cell adhesion molecule (N-CAM)-positive after 15 days of induction (~20 million N-CAM-positive cells at day 15; Figures 1D and 1E), and the proportion of N-CAM-positive cells progressively increased over time (~60 million at day 45, Figures 1D and 1E). On the other hand, the loss of CD45, a hematopoietic specific antigen, along with the downregulation of β2-microglobulin (*B2M*) indicated that reprogrammed cells had lost their hematopoietic identity (Figures 1D and S1D). Importantly, the emergence of CB-iNCs was accompanied by activation of endogenous *SOX2* (82-fold at day 10; Figure 1F), whereas expression of other pluripotency-related genes such as *OCT4*, *NANOG*, *CRIPTO*, *REX1*, and *DNMT3B* (Figure 1G), as well as endodermal/mesodermal-specific transcription factors (*GATA4* and *BRACHYURY*; data not shown), was never detected throughout the conversion process. Likewise, analysis of TRA-1-60 at different time points by FACS analysis showed no positive cells during the conversion process (Figure S1E). These results were consistent in five independent experiments (i.e., five CB units), demonstrating the reproducibility of this reprogramming strategy.

A more extensive quantitative RT-PCR (qRT-PCR) analysis of CB-iNCs confirmed a remarkable upregulation of *TUJ1*, doublecortin (*DCX*), and *MAP2* already at 15 days after neural induction, whereas *PAX6* and early neural markers (*MUSASHI*, *SOX1*, and *NESTIN*) were present at lower levels (Figure 2A). While recent work identified MASH1 as the most important driver of neural conversion in fibroblasts (Chanda et al., 2014), we observed only marginal induction of *MASH1* expression in CB-iNCs (Figure 2A). In contrast, *BRN2* was progressively activated in CB-iNCs during the neural conversion (Figure 2A), suggesting an involvement of *BRN2* in this process, in line with other recent reports (Lujan et al., 2012; Zou et al., 2014).

At this stage, there was little or no expression of mature postmitotic neuronal markers such as *NEUN* (*RBFOX-3*), vesicular glutamate transporter 1 (*vGLUT1*/*SLC17A7*), and *TAU* (*MAPT*) (Figure 2A). CB-iNCs maintained this molecular profile during their propagation (days 15 to 45), when the cells were cultured in the presence of N2 + B27 with fibroblast growth factor 2 (FGF2) and brain-derived neurotrophic factor (BDNF). These data suggest that the conversion process rapidly activates a transcriptional profile characteristic of committed neuroblasts and early neural progenitors.

CB-iNCs Are Expandable and Differentiate into Functional Neurons In Vitro

To confirm the presence of an expandable neural progenitor population, CB-iNCs were disaggregated as single cells and were seeded on matrigel in the presence of N2 media containing FGF2. Under these culture conditions, CB-iNCs displayed neuroepithelial morphology within 1 week (Figure 2Ba) and expressed the early neural marker NESTIN (Figure 2Bb). qRT-PCR analysis also showed upregulation of mRNA levels of early neural progenitor markers (*NESTIN*, *MUSASHI*, and *SOX1*) and concomitant downregulation of neural committed markers (*PAX6*, *TUJ1*, *DCX*,

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